



## **International Certificate Course**

# **“Requisites of seed production, processing, testing and quality assurance”**

(Volume- I)

**20 July 2015 to 20 January. 2016**

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## Seed Quality Control System - Indian Perspective

Agriculture is a way of life in India for nearly seventy per cent of the population and it is the backbone of Indian economy, accounting for approximately 15.7 % of the National Gross Domestic product (GDP). India ranks second only to USA in sheer size of agriculture arable area (140 million ha). By virtue of its large arable land area, sizeable irrigated area, rich agro-biodiversity, diverse agro climate and well-developed research system, the country has all the potential to emerge as a global power in agriculture. The impressive growth registered in agricultural production in general and food grains in particular during the last 50 years have made the country self-sufficient in food grain production with a record of 265 million tonnes during 2012-13. However, India will be home to 1.8 billion populations by 2050, requiring about 430 million tonnes of food grain to feed as per present consumption level ([www.nih.ernet.in/water/htm](http://www.nih.ernet.in/water/htm)). This will pose a big challenge to our Scientists and Planners in the context of population growth and climate change. India has one of the biggest and strong public sector Agricultural Research Systems with ICAR Institutes, State Agricultural Universities and large number of agricultural extension stations and Krishi Vigyana Kendra's (KVKs') spread across the country to develop and deliver the best agricultural solutions to the farmers' needs.

In the significant advances that India made in agriculture in the last five decades, the role of the Seed Industry has been substantial. It is well established fact that the success of green revolution in India was a combination of high yielding varieties of seed and improved fertilizer usage. Globally this is an exciting time to be in agriculture, particularly in the Seed Industry as seed being the foundation of successful agriculture, the demand for quality seeds of improved varieties is growing fast and adoption of new technologies around the world by the farmers is happening at an amazing pace. Therefore, production and supply of high quality seeds of improved varieties to the tiller of the land is a high priority in agricultural growth and development.

### Indian Seed Programme

Among all inputs in agriculture seed is the critical, vital and most important input. India has made significant advance in agriculture in the last four decades, in which role of the seed sector has been substantial. The Indian Seed Industry is currently valued about Rs. 6,500 crores (\$ 1475 Million) and approximately 321 lakh quintals of seeds in volume. There are about 150 - 200 organized seed companies existing in India today. Several companies have recognized Research and Development Units by Government of India and have developed a large number of varieties and hybrids in several crops.

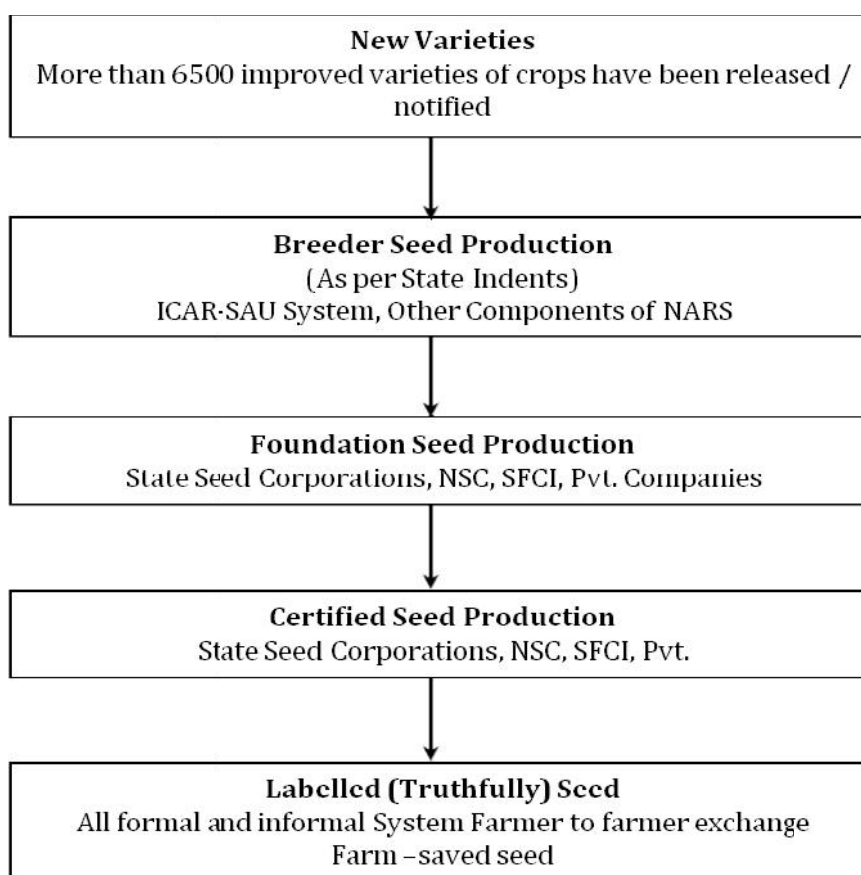
The Indian seed programme is one of the biggest seed markets in the world, with annual sales at around US \$920 million. Of this, domestic off take accounts for US \$900 million and sales in the global market accounts for the remaining US \$ 20 billion. The New Policy on Seed Development (NPSD), established in 1988 with the objective of

augmenting productivity and output quality, stimulated major growth in the Industry as it attracted a lot of investment in seed business from major domestic seed companies.

The present Seed Replacement Rate (SRR) is around 15-20 % for various crops. This SRR level has to be increased to 25 % (proposed 35 %) in self pollinated crops, 33 % in cross pollinated crops (proposed 50%) and 100 % for hybrid crops, in order to increase sustainable agriculture production and productivity for achieving the food, nutritional and social security. Making quality seeds available is going to be one of the most important challenges before us. India has sizeable public and private sector seed businesses. Giant public sector players include the National Seeds Corporation (NSC), the State Farms Corporation of India (SFICI) and thirteen State Seeds Corporations (SSCs). NSC was the first public sector organization, established in 1963. The Central Government is playing major role by extending support to several State Government programmes in seed sectors through Seed Village scheme, capacity building, quality control and extension activities in seeds for creation of Infrastructure and strengthening seed production and marketing of varieties and hybrids of various kinds of seeds.

### Seed Production System in India

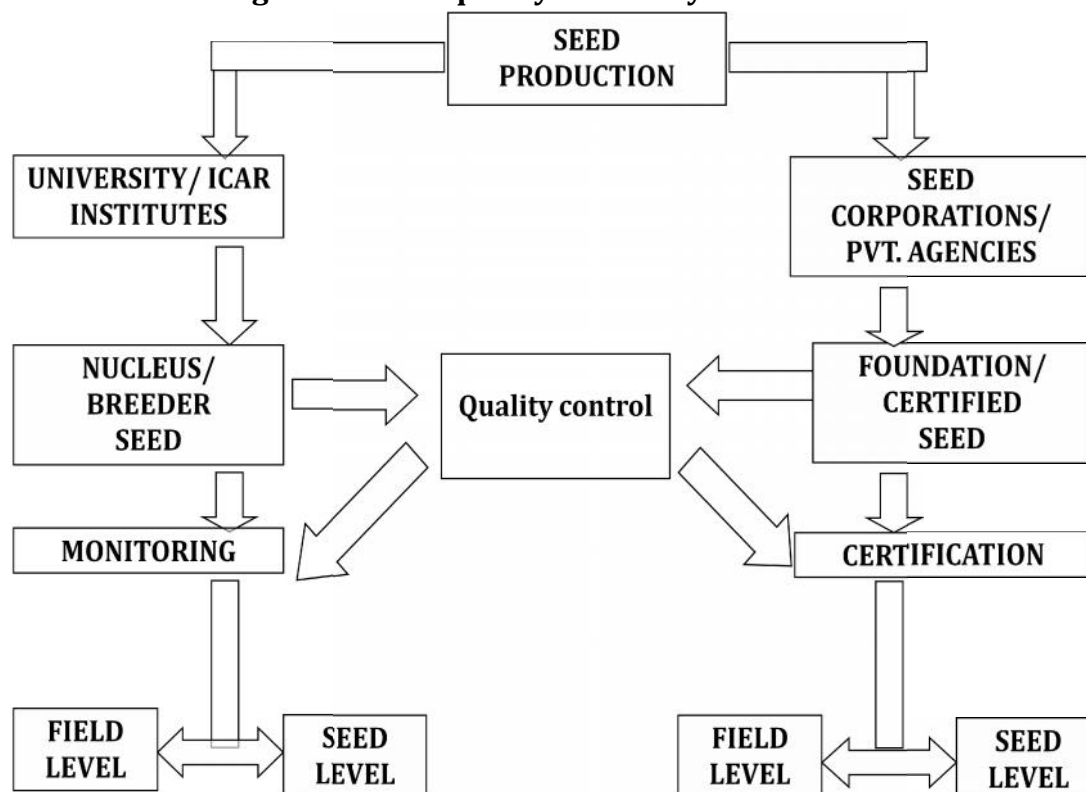
Seed planning generally adheres to the limited seed generation system in multiplication chain in a phased manner. Generally the system recognized 3-4 generations comprising breeder, foundation & certified seeds.



## Seed quality control

Quality control is an important component of the seed programme. A seed programme without the provision of regulating the seed quality control measures may affect badly. There are two aspects of quality control. Firstly the genetic purity of the seed maintained during the production and marketing. Secondly it should have adequate qualities like high Germination and Physical purity, free from weed seeds, disease and have optimum moisture content.

**Fig. 1: Schematic Diagram of seed quality control system in India**



**Seed Regulation:** Seed laws aim to promote varietal and seed quality, thereby 'protecting' farmers from planting sub-standard seed. At the same time, they set the rules of the market for different seed suppliers thus intending to create a 'level play ground'. Seed laws therefore establish the institutional framework of national seed councils and certification agencies and regulate the procedures and standards for:

- **Variety release systems** aim to make only those varieties of proven value available to farmers through the formal seed system.
- **Seed certification** aims to control the varietal identity and purity throughout the seed chain.
- **Seed quality control** checks on other seed characteristics such as viability, purity and seed health. Seed quality control also aims at protecting *bona fide* seed producers from competition by less scrupulous colleagues.

From a legal point of view, seeds are regulated from at least three different perspectives. Firstly, the quality of seeds is regulated to ensure that seeds purchased conform to the characteristics that have been prescribed. Secondly, the safety of seeds is regulated through bio safety measures to ensure that new or imported seeds do not create unwanted environmental harm. Thirdly, in recent years, the intellectual property protection regime has rapidly expanded to include new seeds or new micro-organisms inserted in seeds as products which can be protected under different types of Intellectual Property Rights. The regulation of seeds has been and remains of tremendous importance because of the direct implications that it has on the majority of the population engaged in agriculture as well as on the fulfilment of basic food needs.

The Indian government regulates the Seed Industry and the Seed Trade in various aspects. At present, Indian Seed Industry is governed by Seeds Act (1966), Seed Rules (1968), Seeds Control Order (1983) under Essential Commodities Act-1955, Package Commodities Act (1986), Standards Weights And Measures Act (1976), Consumer Protection Act (1986), The Environment Protection Act (1986), Export Regulation and Quarantine- Plants, Fruits and Seeds (Regulation of import into India) Order (1989), The Destructive Insects and Pests Act (1914), State Acts for the control / movement of crops and seeds (1976), Protection of Plant Varieties and Farmers' Rights Act (2001), Biological Diversity Act (2002), Plant Quarantine Order (2003), Proposed New Seeds Bill (2004), Patents (Third Amendment) Act (2005) and Agricultural Produce Marketing Law (Amendment, 2009).

### **Policy initiatives in Seed Sector**

The important policy initiatives taken by the GOI in seed sector are Seed Review Team (1968), National Commission on Agriculture's Seed group (1972), National Seeds Programme (1974-75), Creation of the Technology Mission on oil seeds and pulses (TMOP-1986) now called as ISOPOM (Integrated scheme of oil seeds, pulses, oil palm and maize), Production and Distribution subsidy, Distribution of Seed Mini-Kits, Seed Transport Subsidy Scheme (1987), New Policy on Seed Development (1988), Seed Bank Scheme (2000), National Seed Policy (2002), Seed Import And Export Policy (EXIM policy, 2002/2007), Formulation of National Seed Plan (2005), National Food Security Mission (2007), and Rashtriya Krishi Vikas Yojana (2007).

### **Mechanism of enforcement**

It is the responsibility of the government to enforce the measures for controlling the quality of the seed being marketed in the country. This can be achieved through legislation in the form of an Act. Government of India enacted the seeds Act in 1966. It is basically regulatory in nature and mainly ensures that seeds of notified varieties offered for sale conform to certain minimum limits of germination and purity. There are two systems in seed quality control and one of which is seed certification and the other is labelling which is compulsory under the seeds Act.

### **The salient features of the Seed Act 1966 are:**

- Regulating the quality of certain seeds for sale and matters connected therewith.
- Constitution of central Seed committee to advise the Central & State governments on the matters arising out of administration of the Act and to carry out the other functions assigned to it under this Act.
- Establishment of Central & State Seed Testing laboratory.
- Establishment Seed Certification Board, Agency and other Committees.
- Investigation and prosecution of the offences under the Act and Rules.
- Implementation of the Act is the joint responsibility of the Central & State Governments.

### **To impose certain penalties for the offences committed under the Act**

Proper implementation of the Seed Act is necessary for maintaining quality and production, distribution, supply, trade and commerce of seeds.

### **Seed Rules, 1968**

The Seed Rules are framed in the year 1968 giving wider scope for understanding various provisions of the Seeds Act 1966. The functions of the Central Seed Testing Laboratory, Seed Certification Agency are elaborately dealt with labelling of any notified kind of variety of seed is made compulsory. It provides certain requirements to be complied with the person carrying on the business of selling seed, these rules are dealt with the following issues;

- Categorized the certified in to three distinct classes.
- Certification procedures are dealt in detail.
- Qualification of seed Analysts and his duties are specified
- Qualification of seed inspectors and his duties are widely defined.
- Procedures for dealing with a written complaint lodged to the seed inspector are laid down.
- Seed sampling procedures are dealt in detail.
- Maintenance of various records and issuing of memorandum in form – VII by Seed inspector to the dealer.

### **Seed Control Order, 1983**

The Seed Control Order 1983 is promulgated under Essential Commodities Act of 1955 mainly with a view to evolve a mechanism for registration of seed dealers, get regular flow of information of seed production and sales and to ensure supply all over the country.

- ❖ This order provides for drawing of samples of seed including truthful labelled seeds.
- ❖ It envisages the obligations on the part of the dealer to obtain incense (clause-3)
- ❖ Display stock board and price list (clause-8)



- ❖ Give memorandum of cash or credit to the purchaser (clause-9)
- ❖ Maintain records and submission of reports (clause-18)
- ❖ Ensure suitable distribution of seeds (clause-10).

#### **It empowers the State Governments to:**

- Call for any information from the dealer (clause-13)
- Cancel the license in case of any violations (clause-15)
- Give directions for regulations of marketing of seed (clause-10)
- To enter upon and search any premises where any seed is stored or exhibited for safe.
- To make samples, seize/confiscate/details seeds stock to ensure compliance.
- Take criminal action against the dealers who contravene the provisions.
- Under this order samples drawn should confirm to the standard of quality claimed on the label.
- It discourages the activities of unscrupulous dealer.

#### **Seed Certification**

Government of India established Certification Agencies for the states under Section 8 of the Seeds Act 1966 and registered under the Societies Act, 1886. At the national level, Central Seed Certification Board h established (1972) under Section-8(a) of Seeds Act, primarily to render advisory services on scientific and operational to the Central Government and State Seed Certification Agencies (SSCA'S). The SSCA'S so established in India discharge their duties as per Section-9 and 10 of Seeds Act. The Section-9 refers to grant or issuance of certificate, Section-10 refers to revocation or withdrawal of certificate issued, based on breach of trust or non - accomplishment of procedure prescribed. The purpose of seed certification is to maintain and make available to public through certification of high quality seeds and propagating materials of notified kind/ variety so grown and distributed, as to ensure genetic identity and purity. Seed certification is also designed to achieve prescribed field and seed standards as per Indian Minimum Seed Certification Standards (Revised in 2013). So far 22 seed certification agencies have been established in India, out of which 17 are independent / autonomous and five are under Department of Agriculture. In India three classes of seeds are being followed viz., Breeder seed, Foundation and Certified seed, out of which only Foundation and Certified seed classes are under the purview of Certification Agency. The seed subjected to certification is labeled. Thus, all certified seed must be labeled, but all labeled seed is not necessarily certified. Agency after confirming all the prescribed field and seed standards issue white and Azure blue (ISI 104) colour tags foundation and certified seed class respectively. While, Truthfully Labelled Seed should carry **Opale green** colour tag.

### **Certification shall be completed in six broad phases:**

- Receipt and scrutiny of application
- Verification of seed source
- Field inspections to verify conformity to the prescribed field standards.
- Supervision of post harvest stages including processing and packing
- Analysis of seed samples including genetic purity & seed health test.
- Grant of certificate and certification tags, tagging and sealing

### **Refusal of Certification**

The Agency shall have the authority to refuse certification of any seed production field or any seed that does conform to the minimum standards prescribed for that particular crop either for field or for seed or for both.

### **Validity Period of the Certificate**

The validity period of the seed lot will be nine months from the date of test at the time of initial certification. The validity period may be further extended for six months provided the seed conforms to the prescribed standards on retesting. A seed will be eligible for extension of the validity period as long as it conforms to the prescribed standards.

### **Appeal**

As per Seeds Act, 1966 there is a provision under Section-11, to make an appeal to an Appellate Authority by aggrieved persons (seed producers and grower), if they are not satisfied with the decision of Certification Agency.

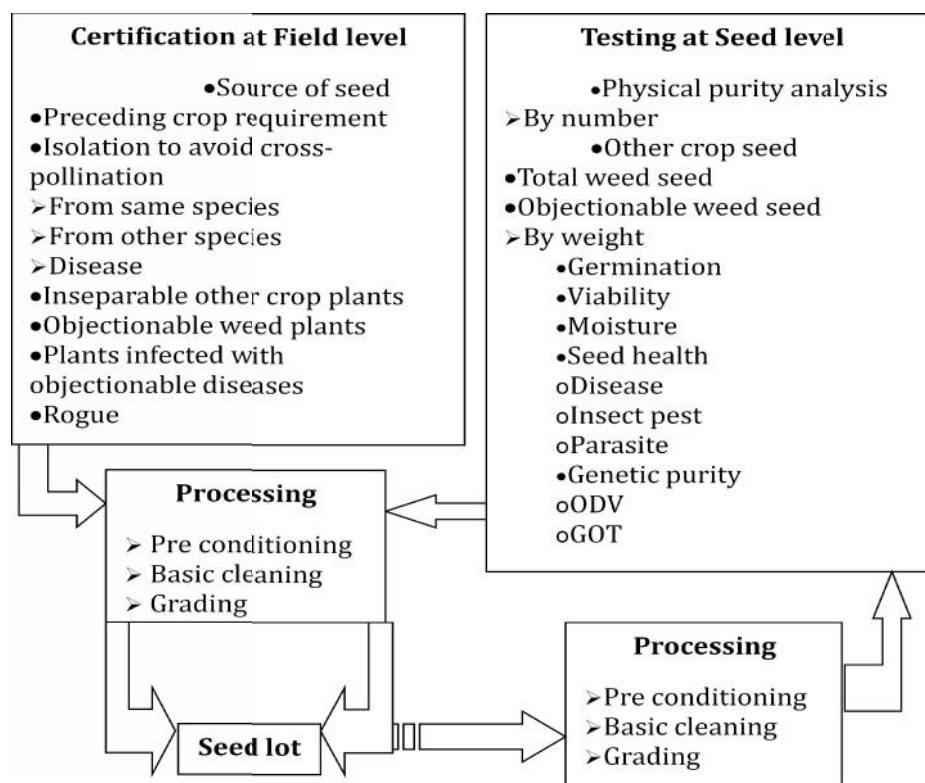
### **Seed Certification Standards**

In India seed certification standards were originally developed by the Central Seed Committee in collaboration with National Seeds Corporation Limited, in the form of Minimum Seed Certification Standards, 1971, which contains General Seed Certification Standards applicable to all the crops and Specific Seed Certification Standards applicable to 56 crops, and since then standards have been amended from time to time. In the year 1988, Indian Minimum Seed Certification Standards were updated which contain Specific Seed Certification Standards for 102 crops and in the year 2013, Specific Seed Certification Standards for 199 crops have been published. The statutory bodies to consider and suggest Certification Standards are:

1. Central sub-committee on crop standards, notification and release of varieties.
2. Central Seed Committee
3. Central Seed Certification Board
4. State Seed Sub-committees

These bodies are constituted for a specific period and are represented by all interests with the seed programme at central / state government level so also private seed enterprises and farmers' representatives.

**Fig 2: Steps of Seed Certification / Seed Quality Testing**



**Enforcement Authority:**

**1. Licensing authority:** the state government may by notification in the official gazette appoints such number of persons as it thinks necessary to licensing authority and may also define in the notification the area within which each licensing authority shall exercise his jurisdiction.

**Application for license:** Every person desiring to obtain a license for selling, exporting & importing of seeds shall make an application in duplicate in Form-A together with a fee of Rs.50/- to the licensing authority.

**Grant of license:** Licensing authority may after making detailed enquiry as it thinks fit grant a license in Form-B, the terms and conditions are enumerated in **Form-B (I to V)**

**The Validity of license:** Every license under this order shall unless previously suspended or cancelled remain valid for three years from the date of its issue.

**Renewal of License:** Every holder of license desiring to renew the license shall before the date of expiry of the license make an application for duplicate to the licensing authority in Form-C together with Rs.20/- fee. If any application is not made before expiry but it is made within one month from the date of the license by paying additional fee of Rs.25/-.

**2. Appointment of seed Inspectors:** The State Government may by notification in the official Gazette appoint such number of persons as it thinks necessary to be inspectors and such notification depend the local area within which each such inspector shall exercise is jurisdiction.

**Qualification of Seed Inspector:** Seed inspector should be a graduate in Agriculture and one year experience in seed production or analysis or development.

**Duties of the seed Inspector:** The seep inspector has to perform all the duties as contemplated under Rule 23 & 23A he shall

- a. Inspect all places used for growing storage or sale of any seed of notified kind/variety.
- b. Satisfy himself that that conditions of the certificates are being observed.
- c. Drawing of seed samples which he has suspected or being produced stocked or sold or exhibited for sale in contravention of the provisions of the act.
- d. Investigate any compliant made to him in writing.
- e. Maintain records of all inspection made and action taken by him in the performance of his duties including the taking of samples and seizure of stock and submit copies of such records. To the director of Agriculture or the seeds certification Agency
- f. He shall detain Imported containers which he has reason to suspect
- g. Institute prosecution in respect of breaches of act and rules.
- h. Performs other duties entrusted by state Govt.

#### **Duties of Seed certification Inspector**

- Verification of seed source.
- Conducting field inspections
- Supervision of seeds processing
- Drawing of seed samples
- Supervision of bagging & tagging.
- Grant of certificate.
- Other duties entrusted by certification Agency.

**3. Seed Analyst:** the State Govt. can appoint the seed analyst. Rule 20 makes it mandatory that the person shall be eligible for appointment as seed analyst, only if he/she posses master

to equivalent degree in Agriculture or Agronomy or Botany or Horticulture of a recognized University and one year experience in Seed Technology.

**Duties of Seed Analyst:**

- On receipt of seed samples for analysis he shall ascertain that the mark and the seal are intact.
- The seed analyst shall analyze the seed samples as per the procedures drawn in the seed testing manual.
- Seed Analyst deliver in form-VII a copy a report of the result of analysis within 30 days from the date of receipt of Samples sent by Seed Inspector.
- Seed Analyst shall forward to the State Govt. The reports giving the results of analytical work done by him.

**3. (a) Appellate Authority for hearing appeals against State Seed Certification Agencies**

**Decisions:**

In exercise of the power conferred under Sub Section (1) of Section 11 of the Act the State Govt. Appoints the Appellate Authority to entertain or render decisions on the appeals filed by the person, refusal, suspension and revocation of certification agency under section 13 & 14 in the matters of grants, refusal, suspension and revocation of certificate.

**Time limit for entertaining appeal:** Sub-Section-1 contemplates that the appeal should be preferred within 30 days from the date on which decision is communicated to him. However the Appellate Authority may entertain an appeal after the expiry of said period if it is sufficient that the appellant was prevented by sufficient cause shown for not filing the appeal in time. Every memorandum of appeal shall be written accompanied by a copy of decision given and treasury receipt of Rs.100/-

**(b) Appellate Authority for hearing cases against refusal/cancellation of seed license**

Any person aggrieved by an order:

- a. Refusing to grant, amend or renew the license for sale, export or import of seeds.
- b. Suspending or cancelling any license, may within 60 days from the date of order, appeal to such authority as the State Govt. May specify on this behalf and the decision of such authority shall be final.

Provided that an application for appeal shall accompany an appeal fee of Rs. 50/-

## **Dealers to comply with Seeds Act & Rules:**

- The holder of license shall from time to time report to the licensing authority any change in his business premises.
- Every dealer of seeds shall display in his place of business.
- Opening and closing stocks on daily basis.
- Price list of different seeds.
- Every dealer shall maintain books, accounts and records relating to his business.
- Dealer shall submit monthly return in his business in form – C to the licensing authority by 5th day of every month.
- No dealer shall sell such seed of notified variety which is false or misleading in any particular concerned in the seed contained in the container.
- Dealer should sell such seed is identifiable of kind/variety.
- Seed conforms to the minimum limits of germination and purity.
- The dealer shall not alter, obliterate or deface any mark or label attached to the container.
- Dealer should not sell or supply after the expiry date of seed stock.
- Dealer should maintain all records of seed sold for a period of three years.
- No dealer should prevent a seed inspector from taking sample any other power conferred on him.
- The dealer shall give every facility to the licensing authority for the purpose of inspection is stock in shop/godown/storage etc.
- The dealer shall display the license at the prominent and conspicuous place in his business premises open to public.

## **2. Legal Measure:**

### **I. Penalties Under Section 19 of the Seeds Act 1966 if any Person**

- a. Contravenes any provision of this Act or any rule made under of
- b. Prevents seed inspector from taking samples under this Act.
- c. Prevents a Seed inspector from exercising any other power conferred on him but or under this Act shall on conviction be punishable.
  1. For the first offence with fine which may extend to Rs.500/-
  2. In the event of such person having been convicted of an offence under section with imprisonment for a term of six months or with fine to Rs. 1000/- or with both.

### **II. Penalties under Seed Control order 1983 (Under essential Commodities act 1955)**

#### **1. If any person contravenes any order made under section-3**

- He shall be punishable

- In case of an order made to clause (h) or (i) of sub-section-(2) with imprisonment for a term which may extend to one year and shall also be liable fine.
  - In case of any other of imprisonment shall not be less than three months but may extend up to seven years and liable to fine.
  - Any property shall be forfeited to the Government.
2. If any person fails to comply with the direction he shall be punishable with imprisonment which shall not be less than three months but may extend up to seven years liable to fine.
  3. If any person convicted under the sub clause – ii of sub-section-I or under Sub-section-2 is again convicted of any offence under the same provision he shall be punishable with imprisonment which shall not be less than six months but may extend up to seven years and liable to fine.
  4. Where a person having been convicted of an offence under sub-section-I is again convicted of an offence under the sub-section for contravention of an order in respect of an essential commodity, in addition to penalty the court may impose that such person shall not carry on any business in that essential commodity for such period as may be specified by court in the order.

#### **Present Problems in seed law enforcement and future needs**

- The existing seed laws in India are being implemented to ensure quality of seed to farmers. However, the degree of success varies from state to state. There are many issues that are considered as impediments for effective implementation of the seed laws. The most important bottle necks are discussed below.
- Presently the country does not have a single and comprehensive act/rule in place to regulate the development and release of crop varieties and their subsequent seed multiplication, processing, marketing and quality check etc, we have several seed laws and associated laws which are either overlapping or inadequate, in other words, not focusing exclusively on seed quality assurance. Hence, there is an urgent need for evolving / enactment of a comprehensive law. There is need for convergence of existing laws which have relevance to seed development, production, distribution/marketing and including import/export.
- Even with the existing laws, implementation has been the biggest causality due to the inherent systems failure / institutional failure. It calls for a review and revision in the seeds standards, seed testing procedures, creation of enforcement authorities and other related laws.
- The quality regulation of vegetatively propagated crop varieties has not received the same attention that has been now given to field crop seeds. It is needless to mention that this needs to be focused in terms of an enactment of suitable laws with appropriated enforcement mechanism.

- Though seed certification is voluntary, there is need to evolve a mechanism adequately supported by law to facilitate certification of all the notified varieties / popular or ruling varieties of both public and private sector.
- The emergence of vibrant private seed industry in the Country through is necessary, has thrown up new challenges in several areas including quality regulation. Hence, the issues relating to public-private partnership, development and release of private varieties and quality related subjects need to be reviewed and revised. There should be a level playing ground provided for both public and private sectors, so much so that farmers interest and national priorities of food security are not jeopardized.
- Establishment of separate enforcement authority/wing in each state for the effective enforcement of existing seed laws / future enactments or comprehensive laws would go a long way in ensuring the availability of quality seeds to the farming community in the country.
- The existing penal provisions do not deter the habitual offenders of law/fly by night operators. Hence, there is an urgent need for enhancing the penalties for contravention of any seed laws / related laws.

#### **New Seeds Bill, 2004:**

The Seeds Bill, 2004 is generally proposed as a replacement for the existing Seeds Act, 1966. The rationale for a new Act can be traced back to the relatively rapid changes that have been taking place in the Seed Sector in the past couple of decades with the adoption of the Plant Variety Act, Biodiversity Act and amendments to the Patents Act which also contributes to following Intellectual Property Protection, participation of MNC's in a big way and the progressive introduction of transgenic seeds.

The stated objective of the proposed law is to "regulate the seed market and ensure seeds of quality". With the proposed changes, the Seed Law would be harmonized with other Seed Laws around the world and ensure the Indian seed market is open to big business. India's New Seeds Bill constituted in 1998, a Seed Policy Review Group in India recommended a long awaited shakeup and reform of the Indian Seed Laws; a new seed law would need to be passed for amalgamating and overcoming the deficiencies in the existing Seeds Act of 1966.

The salient features of the bill are;

1. Making Registration of varieties obligatory.
2. Creation of a National Register of seeds.
3. Regulating (make easier) the imports and exports of the seeds.
4. New regulation on GM crops.
5. Improving the market conditions for private seeds companies.
6. Compensation to farmers.
7. Registration of seed producers, seed processing units, horticultural nurseries.
8. Evaluation of performance of varieties.



9. Accreditation of Seed Certification Agencies.
10. More penalties on offences.
11. Individuals or Seed Producing Organizations to carry out Self Certification.
12. Declaring any Seed Testing Laboratory even under Non-Government sector as a State Seed Testing Laboratory to carry out seed quality analysis.

**Table 1. Comparison between Seeds Act 1966 Vs Seeds Bill 2004**

| Particulars                               | Seeds Act 1966                           | Seeds Bill 2004  |
|---|--|--|
| Coverage                                  | Agriculture & Horticulture               | Agriculture, horticulture, forestry, plantation crops, medicinal and aromatic plants |
| Registration of transgenic varieties      | No provision                             | Special provision  |
| Registration with PVPFR authority         | Not required                             | Required   |
| Period of protection                      | Not defined                              | Defined  |
| Penalty for violation of act              | Rs. 100-1000/- / six months imprisonment | Rs. 5000-5,00000/-one year rigorous imprisonment                                     |
| Self certification                        | Not permitted                            | Permitted  |
| Representatives in central seed committee | From all states                          | From five states only  |
| Involvement of private seed sector        | No                                       | Yes  |

**The genesis of the Indian legislation on Plant Variety Protection (PVP):**

In India, agricultural research including the development of new plant varieties has largely been the concern of the government and public sector institutions. Earlier, India did not have any legislation to protect the plant varieties and, in fact, no immediate need was felt. However, after India became signatory to the Trade Related Aspects of Intellectual Property Rights Agreement (TRIPs) in 1994, such a legislation was necessitated. Article 27.3 (b) of this agreement requires the member countries to provide for protection of plant varieties either by a patent or by an effective *sui generis* system or by any combination thereof. Thus, the member countries had the choice to frame legislations suiting their own system and India exercised this option. The existing Indian Patent Act, 1970 excluded agriculture and horticultural methods of production from patentability. The *sui generis* system for protection of plant varieties was developed by the Indian Government in 2001 by enacting "The Protection of Plant Varieties and Farmers' Rights Act" in the Parliament, integrating the rights of breeders, farmers and village communities, and taking care of the concerns for equitable sharing of benefits. It offers flexibility with regard to protected genera/species, level and period

of protection, when compared to other similar legislations existing or being formulated in different countries. The Act covers all categories of plants, except microorganisms. Further, the GOI passed the Biological Diversity Act in 2002 for protection of biological materials from exploitation. According to this Act, if foreign countries want to utilize our material either for research or trade, they should obtain prior permission from National Biological Diversity Authority of India. The Authority has the head quarters at Chennai, Tamil Nadu. Farmers and community rights are embodied in this Act and give protection to natural materials, but did not deal with the sovereign rights of breeders on their varieties.

### **Objectives of the PPVFR Act:**

- (i) To provide for the establishment of an effective system for protection of plant varieties.
- (ii) To provide for the rights of farmers and plant breeders.
- (iii) To stimulate investment for research and development and to facilitate growth of the seed industry.
- (iv) To ensure availability of high quality seeds and planting materials of improved varieties to farmers.

This Act has 11 chapters and is divided in 97 clauses. The first chapter has title, and the definitions used in context of the Act. The last chapter is on miscellaneous clauses. The other nine chapters deals with PPVFR authority, registration of plant varieties, duration and effect of registration and benefit sharing, surrender and revocation of certificate, farmer's rights, compulsory license, plant varieties protection appellate tribunal, finance, accounts, audit, infringement, offences and penalties, etc.

### **Salient features of the PPVFR Act:**

#### **Authority**

The Central Government shall establish an Authority to be known as the Protection of Plant Varieties and Farmers' Rights Authority (Established in 2005 with head quarter at New Delhi). It consists of a chairperson and fifteen members as representatives of different concerned ministries and departments, seed industry, farmers organizations, tribal communities and State-level women's organization, etc.

#### **Eligibility**

For a variety to be eligible for registration, it must conform to the criteria of novelty, distinctiveness, uniformity and stability (NDUS), as described below [Section 15 (1)-(3)].

For the purposes of the Act, a new variety shall be deemed to be:

(a) *Novel*, if, at the date of filing of the application for registration for protection, the propagating or harvested material of such a variety has not been sold or otherwise disposed of by or with the consent of its breeder or his successor for the purposes of exploitation of such variety (i) in India, earlier than one year.(ii) or outside India, in the case of trees or vines earlier than six years, or, in any other case, earlier than four years, before the date of filing such applications, provided that a trial of a new variety which has not been sold or otherwise disposed off shall not affect the right to protection.

(b) *Distinct*, if it is clearly distinguishable by at least one essential characteristic from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application.

(c) *Uniform*, if subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its essential characteristics.

(d) *Stable*, if it's essential characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle. The variety will be subjected to such distinctiveness, uniformity and stability tests as shall be prescribed.

### **Period of protection**

The certificate of registration issued under section 24 or sub-section 98 of section 23 shall be valid for nine years in the case of trees and vines and six years in the case of other crops, and may be reviewed and renewed for the remaining period on payment of such fees as may be fixed by the rules made on this behalf subject to the conditions that the total period of validity shall not exceed

(i) in the case of trees and vines, eighteen years from the date of registration of the variety;

(ii) in the case of extant varieties, fifteen years from the date of the notification of that variety by the Central Government under Section 5 of the Seed Act, 1996. and

(iii) in the other case, fifteen years from the date of registration of the Variety.

### **Breeders' rights:**

The certificate of registration for a variety issued under this Act shall confer an exclusive right on the breeder or his successor or his agent or licensee, to produce, sell, market, distribute, import or export of the variety [Section 28 (I)].

### **Researchers 'right**

The researchers have been provided access to protected varieties for bonafide research purposes [Section 30]. This Section states, 'Nothing contained in this Act shall prevent (a) the use of any variety registered under this Act by any person using such variety for conducting experiments or research; and (b) the use of a variety by any

person as an initial source of a variety for the purpose of creating other varieties provided that the authorization of the breeder of a registered variety is required where the repeated use of such Variety as a parental line is necessary for commercial production of such other newly developed variety',

### **Farmers' rights**

The farmers' rights of the Act define the privilege of farmers and their right to protect varieties developed or conserved by them [Chapter VI]. Farmers can save, use, sow, re-sow, exchange, share and sell farm produce of a protected variety except sale under a commercial marketing arrangement (branded seeds) [Section 39 (I) (i)-(iv)]. Further, the farmers have also been provided protection of innocent infringement when, at the time of infringement, a farmer is not aware of the existence of breeder rights [Section 42 (1)]. A farmer who is engaged in the conservation of genetic resources of landraces and wild relatives of economic plants and their improvement through selection and preservation, shall be entitled in the prescribed manner for recognition and reward from the Gene Fund, provided the material so selected and preserved has been used as donor of genes in varieties registrable under the Act. The expected performance of a variety is to be disclosed to the farmers at the time of sale of seed/propagating material. A farmer or a group of farmers or an organization of farmers can claim compensation according to the Act, if a variety or the propagating material fails to give the expected performance under given conditions, as claimed by the breeder of the variety.

### **Communities rights**

The rights of the communities as defined, provide for compensation for the contribution of communities in the evolution of new varieties in quantum to be determined by the PPVFR Authority [Section 41 (1)].

### **Registration of essentially derived varieties**

The breeder of the essentially derived variety shall have the same rights as the plant breeder of other new varieties, which include production, selling, marketing and distribution, including export and import of the variety. The other eligibility criteria for award of registration are also the same as for new variety registration under the Act [Section 23(1), (6)].

### **Compulsory license**

The authority can grant compulsory license, in case of any complaints about the availability of the seeds of any registered variety to public at a reasonable price. The license can be granted to any person interested to take up such activities after the expiry of a period of three years from the date of issue of certificate of registration to undertake production, distribution and sale of the seed or other propagating material of the variety [Section 47(1)].

## **Benefit sharing**

Sharing of benefits accruing to a breeder from a variety developed from indigenously derived plant genetic resources has also been provided [Section 26(1)]. The authority may invite claims of benefit sharing of any variety registered under the Act, and shall determine the quantum of such award after ascertaining the extent and nature of the benefit claim, after providing an opportunity to be heard, to both the plant breeder and the claimer.

## **National Gene Fund**

The National Gene Fund to be constituted under the Act shall be credited thereto:

- (a) The benefit sharing from the breeder.
- (b) The annual fee payable to the authority by way of royalties.
- (c) By the compensation provided to the communities as defined under Section 41(1).
- (d) Contribution from any national and international organization and other sources.

The fund will be applied for disbursing shares to benefit claimers, either individuals or organization, and for compensation to village communities. The fund will also be used for supporting conservation and sustainable use of genetic resources, including in situ and ex situ collection and for strengthening the capabilities of the panchayat in carrying out such conservation and sustainable use [Section (45)]. The Indian PVPFR Act thus appears to be an effective *sui generis* system providing a balance between plant breeders' rights along with farmers' rights and researchers' rights. The impact of the Indian *sui generis* system will be felt only after its effective implementation, and later in the areas of research and development, and ultimately in the national food and nutritional security.

Status of the total applications received crop wise up to dated 25.10.2013 were 5384 out of which Public varieties -1183, Private varieties- 2618 and Farmers varieties - 1583

## **International Seed certification and quality control:**

OECD Seeds Scheme for Varietal certification or for the control of seed moving in International Trade. Seed Certification goes hand in hand with seed quality control in which the most important seed qualities -- viability, purity and health -- are tested in a laboratory, commonly using internationally harmonised procedures of the Organisation for Economic Cooperation and Development (OECD) or International Seed Testing Association (ISTA).

## **Back ground Information**

- ❖ The Organization for Economic Co-operation and Development (OECD) an inter-governmental organization founded in 1961, Secretariat at Paris, France provides a multilateral forum to discuss, develop and reform economic and social policies.
- ❖ The OECD's mission is to promote for sustainable economic growth and employment, a rising standard of living and trade liberalization.

- ❖ The OECD brings together its member countries to discuss and develop domestic and international policies during its Technical Working Group and Annual Meetings.
- ❖ It analyses issues, identifies good policy practices and recommends action in a unique forum in which countries can compare their experiences, seek answers to common problems and work to co-ordinate policies.

### **OECD Seed Schemes**

- ❖ The OECD Seed Schemes provide an international framework for the certification of agriculture seed moving in international trade.
- ❖ The schemes were established in 1958 driven by a combination of factors including a fast-growing seed trade, regulatory harmonization in Europe, the development of off-season production, the seed breeding and production potential of large exporting countries in America (North and South) and Europe, and the support of private industry. Membership of the Schemes is voluntary and participation varies.

### **There are seven Agriculture Seed Schemes in OECD viz.,**

- Grasses and Legumes
- Cereals
- Crucifers and other oil or fibre species
- Fodder beet and sugar beet
- Subterranean clover and similar species
- Maize and sorghum
- Vegetables

### **Participating countries**

- ❖ With the recent inclusion of **INDIA, MOLDOVA's and Ukraine** 58 countries from Europe, North and South America, Africa, the Middle-East, Asia and Oceania currently participating in the OECD Seed Schemes.

### **Objectives**

- ❖ The objectives of the OECD Schemes for the varietal certification of seed are to encourage the use of "quality-guaranteed" seed in participating countries.
- ❖ The Schemes authorize the use of labels and certificates for seed produced and processed for international trade according to agreed principles ensuring identity and purity.
- ❖ The Schemes facilitate the import and export of seed, by the removal of technical trade barriers through internationally recognized labels (passports for trade).
- ❖ They also lay down guidelines for seed multiplication abroad as well as for the delegation of some control activities to the private sector ("accreditation").
- ❖ The quantity of seed certified through the OECD Schemes has grown rapidly in recent years and reached 5, 90,000 tonnes.

### How do the Seed Schemes operate?

- ❖ The success of international certification depends upon close co-operation between maintainers, seed producers, trades and the designated authority (appointed by the government) in each participating country.
- ❖ Frequent meeting allow for a multi-stakeholder dialogue to exchange information, discuss case studies prepare new rule and update the Schemes. The UN family of bodies, a vast range of non-government organizations (UPOV, ISTA) and seed industry networks participate actively in the Schemes.

### Benefits of the Schemes

- To facilitate international trade by using globally-recognized OECD labels and certificate.(e.g. they are required to export seeds to Europe).
- To build a framework to develop seed production with counties or companies.
- To participate in the elaboration of international rules for seed certification.
- To develop collaboration between the public and private sectors.
- To benefit from regular exchanges of information with other national certification agencies and observer organizations.

### Rules And Directions Of Oecd Seed Schemes

- ❖ Since 1958, the **OECD Seed Schemes** are open to OECD countries as well as other U.N. Members. 58 countries participate. The OECD certification is applied to varieties satisfying Distinction, Uniformity and Stability conditions, having an agronomic value, and published in official lists. The annual List of Varieties eligible for OECD Certification includes about 42,000 varieties from 194 species.
- ❖ The Schemes ensure the **Varietal identity and purity of the seed** through appropriate requirements and controls throughout the cropping, seed processing and labelling operations. Eg: Generation control (Pre-basic, Basic and Certified seed), isolation distances, purity standards, field inspections, lot sampling, post-control plots, compulsory official laboratory analysis for each certified seed lot.
- ❖ The OECD certification provides for official recognition of "**quality-guaranteed**" seed, thus facilitating international trade and contributing to the removal of technical trade barriers.

### Government of India's Participation in the OECD Seed Schemes

- ❖ The Government of India, Ministry of Agriculture submitted a formal application to the Secretary General of the OECD on 21<sup>st</sup> September 2007, requesting membership of the OECD Seed Schemes. In the application, the Ministry requested to participate in the following seed schemes:
  - Cereal seed.
  - Maize and sorghum seed.
  - Vegetable seed.
  - Grass and legume seed.

- Crucifer seed and other oil or fibre species seed.
- Flower plant material

### Details of the OECD Annual meeting and India's participation

- ❖ The recommendation of the annual meeting for India to join the OECD Seed Schemes (therefore becoming the 56<sup>th</sup> participating country) was forwarded by the Secretariat to the OECD Committee for Agriculture and the council accepted the India's participation in the OECD Seed Schemes during October, 2008.
- ❖ **Kinds of OECD tags / labels are:**
  - Pre-Basic seed – **white tag with a diagonal violet stripe**
  - Basic seed - **White tag**
  - Certified 1st Generation - **Blue Tag**
  - Certified 2nd Generation, or subsequent generations – **Red tag**
- ❖ Not Finally Certified – **Grey Tag** -This is not to be used with the statement "EC rules and standards".

**Table 2. Seed laws in Asian countries at a glance:**

| Country          | Seed Law  | What it Does  | What it Set Up  |
|------------------|---|---|---|
| <b>India</b>     | 1966 Seed Act, amended in 1972 (New Seed Bill, 2004, still to clear Parliament)                           | Regulates the sale of seeds of notified varieties   | Central Seed Committee<br>Central Seed Laboratory and<br>Central Seed Certification Board |
| <b>Korea</b>     | 1970 Major Agricultural Seed Law  | Requires that seeds of eight crops be sold only with a valid seed sale license  | National Seed Council   |
| <b>Indonesia</b> | 1997 Presidential Decree on Seed and 1999 Plant Cultivation Act and its 95PlantSeed Management Regulation | Says that farmers' varieties do not fall under the regulation (they are considered 'natural varieties' and as such, are not controlled by the government) | National Seed Board   |
| <b>Thailand</b>  | 1975 Seed Act revised in 1999   | Prescribes seed labelling requirements and minimum allowable germination requirements for 20 species of seed  | Plant Committee   |



|                    |   |  |   |
|--------------------|---|--|---|
| <b>Pakistan</b>    | 1976 Seed Act (Seed Amendment Bill 2000, still to clear Parliament)     | Prohibits sale, offer for sale, advertising or holding in stock for sale, bartering, or 'otherwise supplying' seed of notified varieties that is not as per prescribed standards | National Seed Council, Provincial Seed Councils, National Registration Agency and Federal Seed Certification Agency |
| <b>Bangladesh</b>  | 1977 Seed Ordinance, followed by Seed Act of 997 and its Seed Rules 998 | Requires that the seed dealer be registered and the seed certified prior to sale for five notified varieties   | National Seed Board, Government Seed Laboratory and Seed Certification Agency                                       |
| <b>Nepal</b>       | 1988 Seeds Act  | Restricts the sale and distribution of seeds without conformity to prescribed standards  | National Seeds Board  |
| <b>Philippines</b> | 1999 Seed Industry Development Act                                      | Promotes the development of the seed industry  | National Seed Industry Council replacing the Philippines Seed Board   |
| <b>Vietnam</b>     | 1996 Decree on the Management of Plant Seeds                            | States that seed producers must be licensed  | Seed Reserve Fund   |

**Table 3. PVP laws in Asian countries at a glance**

| Country          | UPOV Member | PVP Law | Impacts on Farmers   |
|------------------|-------------|---------|--|
| <b>Thailand</b>  | No          | 1999    | Cultivation or propagation from the PVP-protected seed by a farmer may be made not   |
| <b>China</b>     | Yes         | 1999    | The use for propagating purposes by farmers, on their own holdings, of the propagating material of the protected variety harvested on their own holdings shall not require authorization from or payment of royalties to the variety rights holder. Uses other than those mentioned above will |
| <b>Indonesia</b> | No          | 2000    | Allows farmers to use the protected variety as long as not for commercial purposes.  |

|                    |     |                    |   |
|--------------------|-----|--------------------|---|
| <b>Pakistan</b>    | No  | 2000 Ordinance     | Nothing shall affect a farmer's traditional right to save, use, exchange, share or sell his farm produce of a protected variety, except where a sale is for the purpose of reproduction under a branded marketing arrangement   |
| <b>Pakistan</b>    | No  | Draft PVP law 2009 | A farmer shall be deemed to be entitled to save, use, sow, re-sow, exchange, share or sell his farm produce provided that the farmer shall not be entitled to sell seed of a variety protected under this Act on a commercial basis.  |
| <b>India</b>       | No  | 00                 | Farmers can save, use, exchange, share and sell their produce of the protected variety with the restriction that they cannot sell branded seed of the protected variety for commercial purposes.  |
| <b>Korea</b>       | Yes | 00                 | The Minister of Agriculture and Forestry may restrict the breeder's rights to a variety, if a farmer collects the seeds of the variety for himself for the purpose of self-production.  |
| <b>Philippines</b> | No  | 00                 | The traditional right of small farmers to save, use, exchange, share or sell their farm produce of a variety protected under this Act, is maintained except when a sale is for the purpose of reproduction under a commercial marketing   |
| <b>Malaysia</b>    | Yes | 004                | Small farmers can only use seeds of a protected variety on their own field and exchange with small farmers only in 'reasonable amount'. The sale of farm-saved seeds is allowed only in situations where a small farmer cannot make use of the farm-saved seeds on his own holding due to natural disaster or emergency or any other factor beyond the control of the small farmer, and if the amount sold is not more than what is required in her/his |

## **Organising and Implementing an Effective National Seed Quality Control System**

In India, Agriculture is a way of life for nearly sixty percent of the population. The cultivation of land not only sustains their livelihood but also provides a social milieu for their day-to-day living. Accounting for approximately 14.0 % of the National GDP, agriculture is the backbone of Indian economy. Having the largest arable area (140 million ha), India ranks second only to USA in sheer size of agriculture. By virtue of its large arable land area, sizeable irrigated area, rich agri-biodiversity, diverse agro climate and well-developed research system the country has all the potential to emerge as a global power in agriculture. The impressive growth registered in agricultural production in general and food grains in particular during the last 30 years have made the country self-sufficient in cereal grain with a sizeable surplus being regularly exported.

India's' agriculture sector has an impressive long-term record of taking the country out of food shortages with a record food grain production of 254.50 million tones during 2012-13. During the last three decades India's food grain production nearly doubled from 102 million tones during 1972-73 to 200 million tons by 1999-2000. This has contributed significantly in achieving self-sufficiency of food in the country. These figures indicate that agriculture sector has witnessed massive growth on long term basis. However, India will be home to 1.8 billion populations by 2050, requiring about 430 million tones of food grain to feed as per present consumption level ([www.nih.ernet.in/water/htm](http://www.nih.ernet.in/water/htm)). This will pose big challenge to our Scientists and planners in the context of population growth and climate change. However, India has one of the biggest and strong public sector Agricultural Research Systems with ICAR Institutes, State Agricultural Universities, and large number of agricultural extension stations and Krishi Vigyan Kendra's (KVKs') spread across the country to develop and deliver the best agricultural solutions to the farmers' needs.

In the significant advances that India made in agriculture in the last five decades, the role of the seed industry has been substantial. We all are aware that the success of green revolution in our country was a combination of high yielding varieties of seed and improved fertilizer usage. In the green revolution, public sector played a vital role in India with the introduction of high yielding varieties of wheat and rice. Farmers multiplied seed themselves during these days because these were self pollinated crops. This was one of the greatest contributions in spreading the fruits of Green Revolution in India thus making self-sufficient in food from a food deficient country.

The threat of climate change and its impact on agriculture is going to be real. We have some reports that by 2025 in some parts of the world crop yields will drop from anything between 20 and 40 percent from rise in temperatures. The arable land will

become bad that it would no longer be good for cultivation of crops. Hence, world wide it is going to be a real challenge for the agricultural scientists, planners, policymakers. Unless we have advanced technologies, improved crop varieties that can adapt to extremes of weather, we will have difficulty in feeding growing population. Shrinkage of various natural resources, more importantly water is one of the greatest problems is going to face because of climate change.

Globally this is an exciting time to be in agriculture, particularly in the seed industry. Increase in agricultural production is the key to our economic growth. Seed being the foundation of successful agriculture, the demand for quality seeds of improved varieties is growing fast and adoption of new technologies around the world by the farmers is happening at an amazing pace. Therefore, production and supply of high quality seed of improved varieties to the grower is a high priority in agricultural growth and development.

### **Indian Seed Programme**

As we all aware that India is one of the ancient agrarian societies. Nearly sixty percent of the country's population of over 113.9 crores is still dependent on Agriculture. Among all inputs in agriculture production system, seed is the critical and most important input. India has made significant advance in agriculture in the last four decades, in which role of the seed sector has been substantial. The expansion of seed industry has occurred in parallel with growth in agricultural productivity.

The Indian seed industry is currently valued about Rs. 5,750 crores (\$ 1150 Million) and approximately 279 lakhs quintals of seeds in volume. There are about 150 - 200 organized seed companies existing in India today. Several companies have recognized Research and Development Units by Government of India and have developed a large number of varieties and hybrids in several crops.

The Indian seed programme is one of the biggest seed markets in the world, with annual sales at around US \$920 million. Of this, domestic off take accounts for US \$900 million and sales in the global market accounts for the remaining US \$ 6.7 billion. The New Policy on Seed Development (NPSD), established in 1988 with the objective of augmenting productivity and output quality, stimulated major growth in the industry as it attracted a lot of investment in seed business from major domestic seed companies.

The present Seed Replacement Rate (SRR) is around 15-20% for the various crops. This SRR level has to be increased to 25% (proposed 35%) in self pollinated crops, 33% in cross pollinated crops (proposed 50%) and 100% for hybrid crops, in order to increase sustainable agriculture production and productivity for achieving the food, nutritional and social security. Making quality seeds available is going to be one of the most important challenges before us.

India has sizeable public and private sector seed businesses. Giant public sector players include the National Seeds Corporation (NSC), the State Farms Corporation of India (SFCI) and the thirteen State Seed Corporations (SSCs). NSC was the first public sector organization, established in 1963.

The Central Government is playing major role by extending support to several State Government programmes in seed sectors through Seed Village scheme, capacity building, quality control and extension activities in seeds for creation of Infrastructure and strengthening seed production and marketing of varieties and hybrids of various kinds of seeds.

In India we have several organized seed companies and all has recognized Research and Development wing and have developed and released number of varieties and hybrids. All these released and notified varieties and hybrids of various crops should reach the beneficiaries, our farmers within a shorter period as per the need. For that purpose only we are having a National Seed Plan at Central level to monitor and plan for the quality seed production and supply to the various state agencies.

The Government of India enacted the Seeds Act in 1966 to regulate the growing seed industry. The sixties were the most eventful times for Indian agriculture, not only because of introduction of high-yielding cereals, particularly wheat and rice but also for many other positive developments related to seed such as constitution of Seed Review Team, enactment of Seeds Act, 1966 and formation of National Commission on Agriculture. The Seeds Act stipulated that seeds should conform to a minimum stipulated level of physical and genetic purity and assured percentage germination either by compulsory labeling of voluntary certification. Further, the Act provided a system for seed quality control through independent State Seed Certification Agencies which were placed under the control of state departments of agriculture.

India's Seed Industry has grown in size and level of performance over the past four decades. Both private and public sector companies/ corporations are involved with the production of seed. The seed sector in India has witnessed rapid changes since liberalization. The industry has made impressive strides from a modest beginning in seed production and quality seeds distributed in the country increased from 1.83 lakh quintals in 1953-54 to 93.95 lakh quintals in 2001-02. The projected seed requirement by 2020 AD is estimated at 320 lakh quintals by considering the proposed enhancement of seed replacement rates.

Further, our agricultural systems are moving very fast to cope up with the advancement in technology transformations and hence we should equip and create the infrastructural facilities and trained man power on a par with science advancement, so as to produce and supply good quality seeds to our farmers. This could be done through continuous capacity building programme in which all the latest technologies are need to

be transferred to our officials and seed producers in order to update their skill and knowledge. Then only the our prime objective and vision of timely supply of quality input materials in agriculture in order to increase our agricultural productivity, food grain production and more importantly to make our farmers to share the benefits of new varieties and technologies.

For that purpose only, the Government of India, Ministry of Agriculture has established the National Seed Research and Training Centre (NSRTC) at Varanasi, is a centre for Seed Quality Control and training human resources on total seed quality management and regulation. The NSRTC is equipped with all state-of art facilities and it is the model seed testing laboratory for the nation. Further, the NSRTC has already become the Member Laboratory of the International Seed Testing Association (ISTA), during 2007.

More importantly, the NSRTC is the apex human resource training centre at central level, providing a forum to all the stake holders of seed industry and seed quality control personnel to present their views on critical issues relating to seed, seed production and quality control by organizing National and International seed related brainstorming sessions, workshops, seminars, symposia and seed science congresses every year by involving experts and stake holders to deliberate on issues of current importance and brings out policy documents on seed quality control for use of scientists, planners, decision makers and seed industry to sustain quality seed availability and distribution to the farmers which in turn make agricultural growth to attain evergreen revolution in the country.

### **Concept of seed quality**

All those seeds are expected to be of highest quality. That means, the seed lot shows

- Highest purity and is free of noxious weeds
- The germination is high, seeds germinate quick, even and simultaneous
- The vigour of the seeds is high to perform well also under unfavourable conditions in the field
- The seeds are healthy or properly treated to avoid contamination with or spread of pathogens such as fungi, bacteria, viruses, nematodes or insects and mites
- The seeds conform to the cultivar claimed for to avoid failure during production and in the final product. This is valid for varieties of traditional breeding as well as for those genetically modified.
- The moisture content should be of appropriate level to avoid any decay of the seed.

### **Genetic Purity**

This is the most important seed quality attribute. The genetic purity of the seed lot governs the yield potential of the variety. It is, therefore, very important that the genetic (cultivar) purity status of the seed lot should be high. In a seed certification

scheme, the genetic purity standards have been prescribed and Genetic purity of the breeder seed should be maintained through maintenance breeding programme.

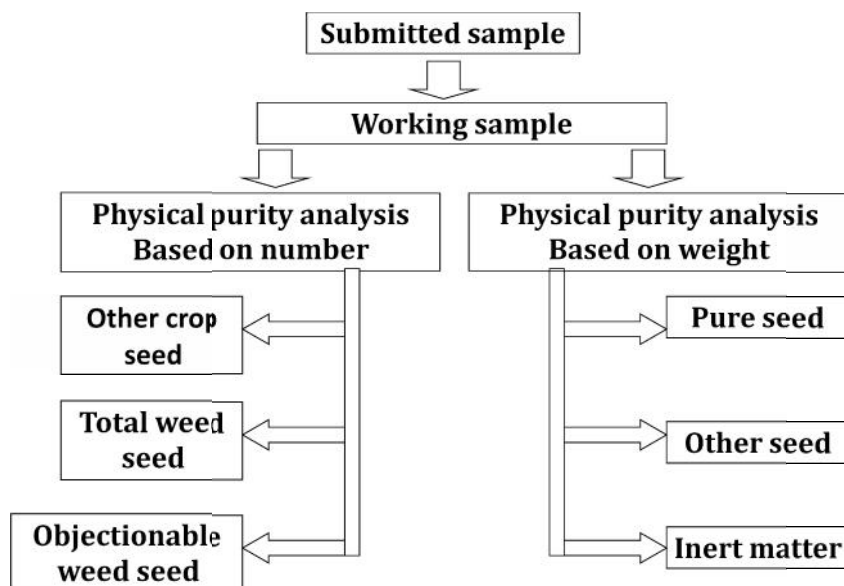
Genetic purity is controlled in the field through the process of seed certification. For maintaining genetic purity, generation system of seed production i.e., Breeder to Foundation and Foundation to Certified, has been evolved. Genetic purity can be tested in the field-plot test and through various biotechnological tools.

### Physical Purity

The physical purity of the seed lot should also be high. The seed, which is obtained after harvesting and threshing the seed lot, is not fit for immediate sowing or planting purpose. It contains certain admixtures, such as soil, stone pieces of leaves, barks, chaff and other foreign material together with seeds of other foreign material together with seeds of other crops and weeds. The occurrence of these admixtures reduces the planting value of the seed lot because these admixtures are undesirable as these may reduce the yielding ability of the seed lot.

Seed is a basic input in modern agriculture. It has been an important agricultural commodity since the first crop-plant being domesticated. A farmer's entire crop depends on the quality of the seed he sows. Therefore, it is necessary to plant good quality seed. Seed quality is a concept made up of several attributes. The quality seed also helps primarily for the environmental development and hence SEED can be called as Scared Entity for Environmental Development.

**Fig. Physical Purity Analysis**



## **Germination**

Germination percentage indicates the potential of seeds for developing and establishing into seedlings. Germination capacity of the quality seed lot should be high for obtaining the desired crop stand in the fields. Using seeds of low germination will reduce the field establishment or stand and thus the yields will be lowered. Seed germination is affected by a variety of factors, which are imposed to the seed during its formation, maturation, ripening, such as, infection with pests and pathogen, sub-optimal conditions of nutrients and water supply and untimely rains or frost at the maturity stage. In addition, post-harvest operations and handling of the seed lots during marketing or distribution are also responsible for affecting the seed quality.

## **Moisture**

The life processes of the seeds revolve around the seed moisture content. The moisture content of the seeds should not be too high or too low. Seed moisture content should be brought to the desirable level, to preserve their viability in storage and avoiding the spoilage by insects and pathogens. Seed-moisture content is also important for seed processing. Seeds should possess optimum moisture while processing to avoid damage to living embryo; when the moisture content is more or less than the optimum, the seeds get damages. The moisture content of the seeds varies according to its chemical composition. It is less in case of oily seeds while it is high in case of those seeds where reserved food material is either predominantly carbohydrate or proteins.

## **Seed Health**

Seed health is an important seed quality attribute, especially under tropical and subtropical conditions. Quality seed should be free from seed-borne diseases and insect infestation. Insect infestation normally destroys embryos thus making the seeds unfit for sowing. Seed-borne diseases may be controlled by chemicals, but the chemicals used may be poisonous to men and animals. To avoid diseases a better policy is to sow seeds harvested from healthy crops, and to achieve this, seed certification schemes fix standards for health which should be followed.

## **Seed Vigour**

The performance potential of a seed lot with reference to field establishment is very much dependent on the capacity of the seed to germinate, emerge and establish under sub-optimal field conditions. This performance potential or 'hidden stamina' of the seed makes it fit to perform well upon sowing is called seed vigour. It indicates the ability of seed to emerge in varying environments of fields. It is generally believed, but not always true, that high germination percentage is associated with high vigour.

## **Size and Uniformity**

Uniformity of size is important for sowing by machines and for precision planting. It is believed that large size seeds yield more than smaller seeds, but there are



many exceptions to this rule. However, shriveled seeds should be removed during cleaning and processing.

## **Seed Reforms, Planning & Legal Aspects of Quality Regulation**

### **Developments in India**

The important milestones in seed planning and reforms are as under.

- ❖ Royal Commission on Agriculture constituted in 1926. Recognised the importance of quality seed distribution for improving agriculture production.
- ❖ Introduction of high yielding and dwarf varieties of wheat and rice and hybrids in maize, sorghum and pearl millet in 1960's.
- ❖ 1961- 1<sup>st</sup> Seed Testing Laboratory established in India.
- ❖ Setting up of National Seeds Corporation (NSC) in 1963 heralded systematic production of improved seeds.
- ❖ Establishment of 'State Farms Corporation of India' (SFCI) in 1969.
- ❖ GOI passed the Seeds Act (1966) and framed Seed Rules (1968).
- ❖ Establishment of State Seeds Corporation and State Seeds Certification Agencies in the decade of 1970s.
- ❖ Seed Control Order (1983) was passed to control the persons involved in seed business through the compulsory licensing
- ❖ Relaxation of the restrictions on importing of seed through New Seeds Policy (1988)
- ❖ Environment & Protection Act 1986 & its Rules 1989.
- ❖ Proposal of "New Seeds Bill 2004" – combining Seeds Act (1966), Seed Control Order (1983) and New Seed Policy (1988)

Seed planning generally adheres to the limited seed generation system in multiplication chain in a phased manner. Generally the system recognised 3-4 generations comprising breeder, foundation & certified seeds.

### **Seed quality control**

Quality control is an important component of the seed programme. A seed programme without the provision of regulating the seed quality control measures may affect badly. There are two aspects of quality control. Firstly the genetic purity of the seed maintained during the production and marketing. Secondly it should have adequate qualities like high Germination and Physical purity, free from weed seeds, disease and have optimum moisture content.

### **Mechanism of enforcement**

It is the responsibility of the Central and State Governments to enforce the measures for regulating the quality of seed being marketed in the country. This can be achieved through legislation in the form of Act. There are two systems in seed quality control and one of which is Seed Certification and the other is Labelling. Seed certification is voluntary and labelling is compulsory under the Seed Act.

## **Indian Seed Act 1966 - salient features**

To control the quality of seed during production and marketing, Government of India enacted Seed Act in 1966 and framed the Seed Rules in 1968. It is basically regulatory in nature and mainly ensures that seeds of notified varieties offered for sale, conform to certain minimum limits of germination and purity.

The Seed Act was implemented in 1968. For effective implementation of the act, major provisions are:

- Regulating the quality of certain seeds for sale and matters connected therewith.
- Establishment of Central Seed Committee
- Minimum Seed Certification Standards, 1971
- Certification voluntary but labeling is compulsory
- Label should contain necessary information's
- Establishment of Central and State Seed Testing Laboratory
- Establishment of Central Seed Certification Board
- Establishment of State Seed Certification Agencies
- Provision of Seed Inspectors and Seed Analysts
- Law Enforcement
- Investigation and prosecution of the offences under the Act and Rules.
- Implementation of the Act is the joint responsibility of the Central & State Governments.
- To impose certain penalties for the offences committed under the Act.

The proper implementation of the Seed Act is necessary for maintaining quality during production and distribution.

## **Seed Rules 1968**

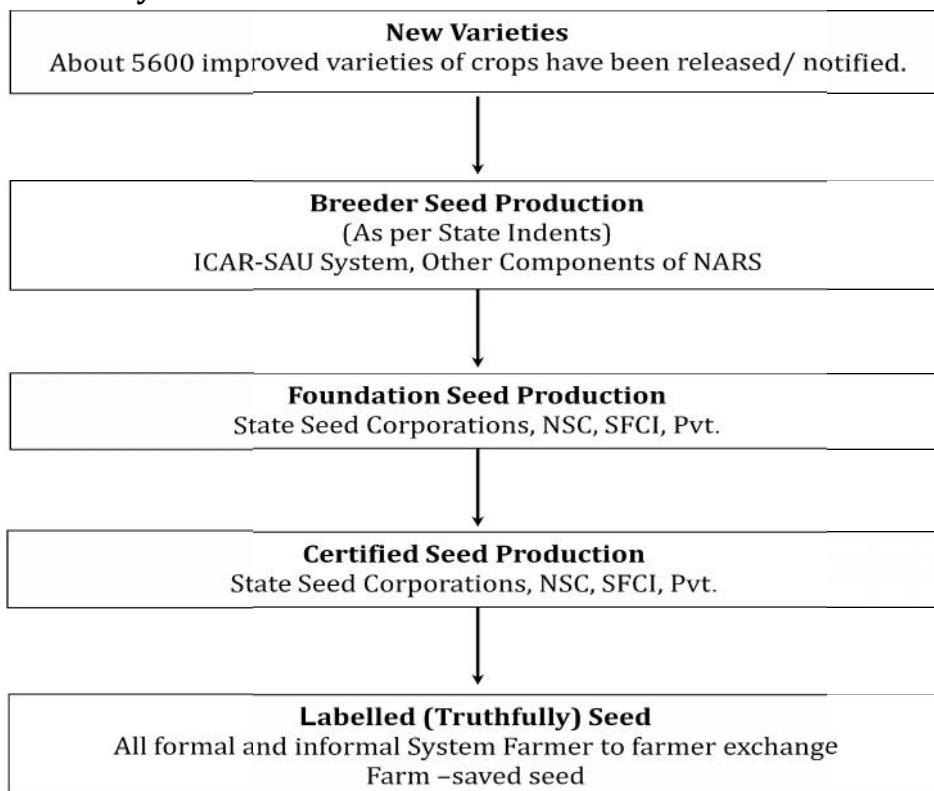
The Seed Rules are framed in the year 1968 giving wider scope for understanding various provisions of the Seeds Act 1966. The function of the Central Seed Testing Laboratory, Seed Certification Agency are elaborately dealt with. Labeling of any notified kind of variety of seed is made compulsory. It provides certain requirements to be complied with the person carrying on the business of selling seed these rules are dealt the following issues;

- ❖ Categorized the certified in to three distinct classes.
- ❖ Certification procedures are dealt in detail.
- ❖ Qualification of seed Analysts and his duties are specified.
- ❖ Qualification of seed inspectors and his duties are widely defined.
- ❖ Procedures for dealing with a written complaint lodged to the seed inspector it laid down.
- ❖ Seed sampling procedures are dealt in detail.
- ❖ Maintenance of various records and issuing of memorandum in Form-VII by Seed Inspector to the dealer.

### Indian Minimum Seed Standards, 1971

- These include standards both field level and seed level
- These standards were revised in 1988 along with inclusion of more crops

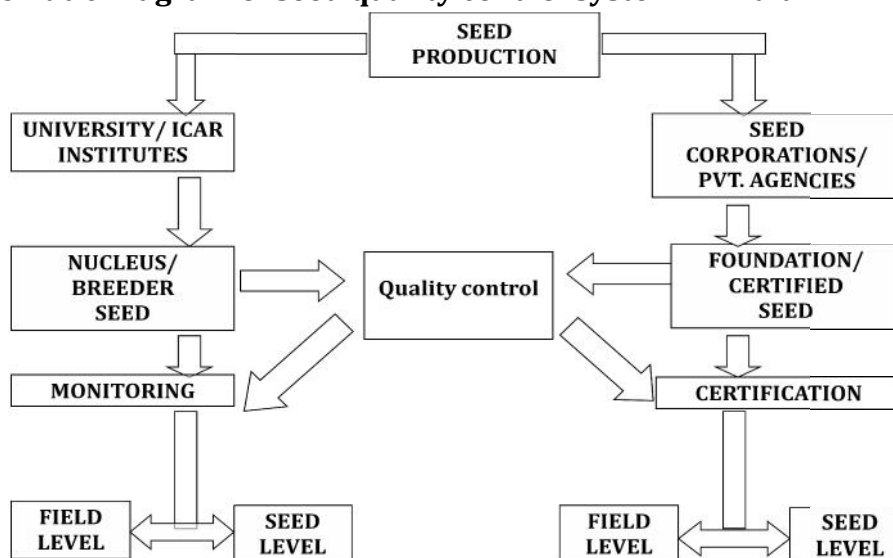
### Seed Production System in India



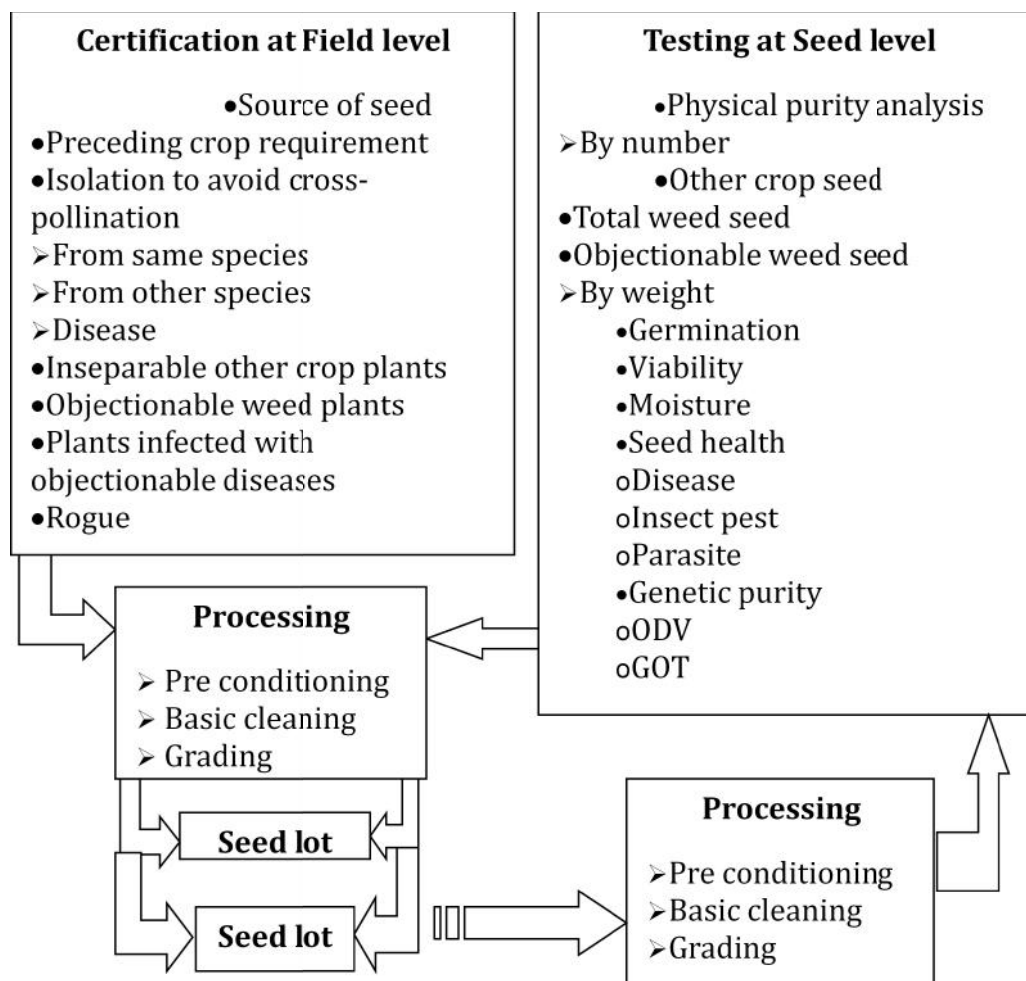
### Our Concerns are:

- Total quantity of seed / seed produced per unit
- Quality of seed

**Fig. 1: Schematic Diagram of seed quality control system in India**



**Fig 2: Steps of Seed Certification / Seed Quality Testing**



**Parameters of Development in Seed Quality Regulation**

- Existence of National Seed Policy
- Seed legislation
- Existence of Variety Development & Variety Release system
- Variety Release committee
- Seed quality control and certification
- Official seed testing laboratories
- Trained inspectors and lab technicians - HRD

**Seed Law Enforcement for Transgenic Crop Species including Bt Cotton**

The biotechnology in agriculture covers a wide range of techniques to improve productivity and quality in crops. Several biotechnology applications such as plant tissue culture (micro propagation), bio pesticides, bio fertilizers, diagnostics for diseases of crops and livestock, embryo transfer in livestock have, already been adopted extensively in India. New recombinant DNA or genetic engineering technologies are now being used to transfer genes within and across plant species to generate genetically

modified organisms (GMOs) including transgenic crops. Whereas the conventional biotechnology products and processes are simple to use, often inexpensive and relatively free of regulation, the transgenic crops involve advanced skills and research inputs and are highly regulated.

Transgenic crops incorporate traits such as herbicide tolerance, pest and disease resistance, product quality improvements and stress tolerance. Over 15 transgenic crops have come into commercial use world over.

In India, the first transgenic crop (Bt cotton) was approved for commercial cultivation on 26<sup>th</sup> March 2002. Due to the positive effects the farmers got from Bt cotton, the area under transgenic cotton doubled from 45000 ha in the year 2002 to 0.1 million hectares in 2003 and reached 7.6 million hectares in 2008-09 constituting nearly 81% of the total cotton area in India with 621 Bt cotton hybrids and varieties approved till December 2009. Considering this pace of growth, the demand for GM seeds too, will in turn, increases exponentially. In the post WTO era, the free access to international seed market, will also promote the movement of GM seeds across the borders.

As more and more transgenic crops are being released for field testing and commercialization, concerns have been expressed about the potential risks associated with their use to human health, environment and biological diversity. Questions are continuously raised about risks such as unintended changes in characteristics of the exposed species, the possibility of adverse impact on non target species, the potential for weediness in genetically modified crops and the stability of the inserted gene.

To address the above concerns, bio safety regulations have been put in place by many countries involved in transgenic research and commercialization. The Cartagena Protocol on Bio safety has also come into force on September 11, 2003 that governs trans boundary movement of GMOs. As on December 31, 2003, 78 countries have ratified the protocol including India. India has a well defined regulatory mechanism for development and evaluation of GMOs including transgenic crops and the products thereof. Rules notified in 1989 under environmental Protection Act, 1986 (EPA) define the competitive authorities and composition of such authorities for the handling of all aspects of GMOs and products thereof. Presently, there are six competent authorities, a Recombinant DNA Advisory Committee (RDAC), a Review Committee on Genetic Manipulation (RCGM), Genetic Engineering Approval Committee (GEAC), (apex bodies), Institutional Bio safety Committees (IBSC) attached to every organization engaged in rDNA research, State Bio safety Coordination Committees (SBCC) and District Level Committees (DLC). Guidelines for safety in biotechnology have been issued by the Department of Biotechnology (DBT) in 1990 covering research, field trials and commercial applications. DBT also brought out separate section on transgenic plant varieties.

Transgenic Crops or Genetically Modified Crops introduced in India are governed by

1. Environment (Protection) act 1986
2. Rules for the Manufacture, Use, import, Export and Storage of Hazardous Micro organisms Genetically Engineered Organisms or Cells.

### **Seed Testing Network in India - Central Seed Testing and Referral Laboratory**

The Central Seed Testing Laboratory was established in the erstwhile Division of Botany, IARI, New Delhi in 1961 with the aid of Rockefeller Foundation as the Nodal Seed Testing Laboratory. In 1968, there were only 23 Seed Testing Laboratories and at present we have 108 State Seed Testing Laboratories in the country.

At present, the Government of India, Ministry of Agriculture has established the National Seed Research and Training Centre (NSRTC) at Varanasi, to have an exclusive National Seed Quality Control Institute as Central Seed Testing and Referral Laboratory. The institute is also offering human resource trainings for scientific and technical personnel engaged in seed testing and certification on seed quality management and regulation. The NSRTC is equipped with all state-of-art facilities and it is the model seed testing laboratory for the country. The Central Seed Testing Laboratory (CSTL) undertakes analysis of seed samples under Seeds Act 1966, standardization of seed testing procedures, uniformity in seed testing results among different State Seed Testing Laboratories.

In 1968, there were 23 Seed Testing Laboratories in the country. At present one Central Seed Testing Lab and 108 State Seed Testing Laboratories are operating in the country. 11 laboratories in the country (5 in public and 6 in private sector) are members of the International Seed Testing Association (ISTA). Of these, 5 in the private sector have obtained accreditation of ISTA to issue certificates. Three more public sector ISTA member laboratories are in the process of being accredited for issuance of International analysis certificates. A well equipped and well-staffed laboratory can serve the needs of both the certification and quality control programmes, and that of the cultivators and dealers. It can also conduct the research in solving the practical problems in seed testing.

### **Central Seed Testing Laboratory (CSTL)**

The important functions of analyzing the litigation seed sample (seed samples referred by the court of law) and service samples by Central Seed Laboratory has been indicated in the Act and Rules. In addition to the function entrusted to the Central Seed Testing Laboratory by the Act, the laboratory shall carry out other functions, namely, a) initiate testing programmes in collaboration with the State Seed Laboratories designed to promote uniformity in test results between all seed laboratories in India, b) collect data continually on the quality of seeds found in the market and make this data available to the Committee, and c) carry out such other functions as may be assigned to it by the Central Government from time to time.

The Central Institute for Cotton Research (CICR), ICAR, located at Nagpur, is the Referral Lab for testing *Bt* cotton. The institute has developed three kits for testing *Bt* cotton at three levels. These are *Cry 1 Ac Bt*- Quant, an ELISA Kit, which facilitates a precise quantification of *Cry 1 Ab* or *Cry 1 Ac*, expressed in transgenic plants.

### **State Seed Testing Labs (STLs)**

State Seed Testing Laboratories are meant to analyse the seed samples of any notified kind or variety received from various sources for the following purposes viz., a) analysis of the samples received from Seed Certification Agencies set up under Section 8 of the Seeds Act, b) analysis of the samples from seed users and seed producers who could get their seed samples tested to obtain the result to be used as information for seeding, selling or labelling purpose and, c) analysis of the samples received from Seed Inspector to determine the compliance of labelling requirements under Section 7 of the Seeds Act.

### **State Seed Certification Agencies (SSCAs)**

The State Seed Certification Agencies (SSCAs) are responsible for seed certification in the concerned states. In India, 20 State Seed Certification Agencies are involved in the regulation of quality seed production and its distribution. The SSCAs perform several functions viz., a) they screen the applications from seed growers for seed certification and decide on their fitness, b) they also check and verify the appropriateness of the source seed used for growing the seed crop under certification, c) they carry out the requisite field inspections, d) they conduct the seed tests, e) they certify the seeds found suitable and issue the appropriate tags both for certified and foundation seeds, f) they guide the seed growers on production, processing and distribution of seeds, g) they conduct short courses on seed production, etc. for seed growers, and h) they participate in other activities conducive to the development of seed industry, e.g. preparing and publishing lists of plant breeders, seed growers, etc.

### **Certification Requirements with Respect to Seed Testing**

The object of the seed certification is to maintain and make available to the public high quality seeds and propagating material of notified kind/varieties so grown and distributed as to ensure genetic identity and genetic purity. The certification standards in force (IMSCS) and seed certification procedures, together form the seed certification regulation. Seed of only those varieties or kinds, which are notified under Section 5 of the Seeds Act, shall be eligible for certification.

To ensure the quality seed material, field standards during seed production are most important. The seeds so produced should meet the prescribed standards for germination %, genetic and physical purity and seed health in some cases. The standards, so prescribed should be achievable by the grower and at the same time high enough to meet the needs of the farmer.

All seed lots should conform to the minimum standards for genetic purity, which for foundation class of seed is 99.0%, certified seeds of varieties, composites, synthetics, and multilines it is 98.0% and for hybrids the minimum prescribed standard of genetic purity is 95.0% except cotton, watermelon, brinjal, muskmelon, and tomato, which should possess 90% of genetic purity. In case of castor hybrids it will be 95.0% and 85.0% for Foundation seed and certified seed, respectively.

### **Grow Out Test (GOT)**

It is the recommended procedure for determining/testing the genetic purity of a notified seed lot. It may serve both as a 'pre-control' and 'post-control' test for avoiding genetic contaminations. According to the official regulations in India, it is a pre-requisite for seed certification of hybrids of certain species such as cotton, castor, musk-melon and brinjal which are produced through manual operation of emasculation and/or pollination or by using CHA. The test may also be conducted for checking the seller's label with respect to varietal purity status of the seed lot under the provisions of the Seeds Act, 1966. In addition, Grow Out Test can also be used as a measure to judge the efficacy of the Inspector of the Certification Agency.

### **Laboratory Tests for verification of species and cultivars**

Identity of a cultivar is established by means of heritable characters (morphological, physiological, chemical etc.). Different laboratory methods are available, which are based on examination of morphological seed characters, colour reaction to certain chemical treatments and seedling responses (ISTA, 1993). Variability in the colour reaction of seed subsequent upon application of chemicals provides a simple method to group a large number of genotypes into distinct classes to differentiate between genotypes. The phenol colour reaction is used in varietal purity determination in a number of crops, namely, wheat, barley, oats, rye, grass, Kentucky blue grass and paddy. Peroxidase test is one of the standard tests, which is used in differentiating soybean cultivars.

### **The Back ground GM Seed Detection**

Seeds will be the primary delivery system for the transgenic technology in the majority of annual crops. However, "value-added" traits derived from genetic engineering are only valuable when added to elite cultivars already possessing high yield, disease resistance, and quality traits that make them competitive in the market. This value addition such as crop protection (herbicide resistance, insect resistance, disease resistance) and improved end-product quality, will in turn lead to increase in the cost of GM seeds. This situation of huge market demand and the high profit margins involved in the GM seed trade, will create a congenial atmosphere in seed markets for the sale of spurious seeds and admixtures of GM/non-Gm seeds, which is already prevailing in states like Gujarat and



Maharashtra. On the other hand due to the risks associated with the use of transgenic seeds (*viz.*, spread of transgenes to non-transgenic crops, development of super weeds & pest resistance, potential impact on human & animal health and threat to plant biodiversity) many countries have approved only a few of the GM crops.

- To ensure the varietal purity of GM seeds with respect to expression of inserted transgene
- To detect and quantify the adventitious contamination of GM seeds in conventional seed lots
- To prevent the entry of unapproved transgene event in to the country along with the imported seed/food material
- To certify that the seeds used in organic farming are free from GM seeds or its contamination
- To enforce the proper labeling of GM products and food materials
- Verification of Varietal Purity of GM Seeds -Detection of GMO in Non-GM Seed Lots through
  - a. Detection
  - b. Identification
  - c. Quantification

### **Seed health test**

Though the fungal plant pathogens are the most pre-dominant ones, it is interesting to note that seeds often carry some saprophytic fungi or bacteria which may produce a toxic substance which may control some important plant disease, e.g. *Fusarium nivale* causing seedling blight in oats may be controlled by species of *Chaetomium* found associated with oat seeds. Similarly, the seed transmitted virus diseases are very important, particularly in legumes and vegetables. Certain seed borne disease need longer period for their expression than provided in the normal incubation tests. The pathogens are identified based on symptoms followed by tests of infectivity/electron microscopy/ELISA in case of viruses.

### **Advanced technologies for seed health testing**

It is a well established fact that seeds are both victim and vehicle of the extensive and complex micro flora. Some of the most important plant pathogens are seed borne and/or seed transmitted. The various disease causing organisms *i.e.* fungi, bacteria, viruses and even nematodes are carried with the seeds. In addition to microscopic and histochemical examination, which can detect and identify a large number of fungi, more precise diagnostic techniques are to be used for bacteria, viruses and some fungi *viz.*,

### **Serological methods**

Enzyme Linked Immunosorbant Assay (ELISA), Dot-Immuno Binding Assay (DIBA) and Immunosorbant Electron Microscopy (ISEM) provide precise tools for the detection of seed borne viruses.

## Molecular Methods

Polymerase Chain Reaction (PCR) is most sensitive technique for detecting plant viruses, bacteria and fungi even at a low level of incidence. These techniques are most useful in detecting strains which are new or morphologically similar to known pathogenic strains.

### International Certification

- International certification differs from national certification, as domestic regulatory systems may vary to a large extent.
- A voluntary international system is a tool which heterogeneous countries can use for specific product characteristics, without having to change their domestic framework.
- The benefits from certification and guarantees are shared between all stakeholders; consumers, producers, industry, exporters and importers.

### Other Seed Certification Systems

- United States, for example, seed certification is not mandatory.
- Association of Official Seed Certifying Agencies (AOSCA), and this is done on a voluntary basis. AOSCA promotes and facilitates the movement of seed in local, national and international markets.
- European Union (EU) "marketing of seed (including seed potatoes) is regulated by Directives agreed and implemented by all 27 Member states"

### Organization for Economic Cooperation and Development (OECD) and International Seed Testing Association (ISTA);

OECD: Varietal Certification to Ensure Varietal Identity & Purity for International movement / Trading of Seeds.

- ISTA: Ensure seed quality standards by globally harmonized Sampling & Testing procedures
- Issue of Seed Analysis Certificates to facilitate International trading / movement.
- In India : 22 member ISTA laboratories  
4 ISTA accredited laboratories

Though these are not directly involved in seed certification but promote International seed trade. These are

1. International Seed Testing Association (ISTA): Develops accurate and uniform methods for testing and evaluating seeds.
2. Organization for Economic Cooperation and Development (OECD): Develops Certification System acceptable for member countries.
3. International Crop Improvement Association : It is an organization of Seed Certification Agencies in USA and Canada which was changed to the Association of Official Seed Certifying Agencies (AOSCA) in 1969 and responsible for ;
  - To establish minimum standards for certification.
  - To promote production, identification and distribution of seed.

- To educate growers.

Good quality seed acts as catalyst for realizing the potential of all other inputs in agriculture. Hence, high quality seed is as important as an improved variety.

Currently there is a virtual revolution in the area of seed quality and seed industry. It is not enough to have a desirable cultivar, quality seed must accompany cultivar improvement or the cultivar will never succeed in the market place. Today's seed consumer is well aware of the implications of seed quality has on crop establishment, yield productivity and cost.

Dr. M. S. Swaminathan writes "Rewards from recent advances in breeding techniques as well as from the opportunities now available for producing novel genetic combinations, will be proportional to advances in Seed Technology".

### Seed Informatics and Communication (SeedNet)

(A Step towards Seed Informatics on-line  
for Sustainable growth in Agricultural production)  
<http://seednet.gov.in>

#### SeedNet India Portal

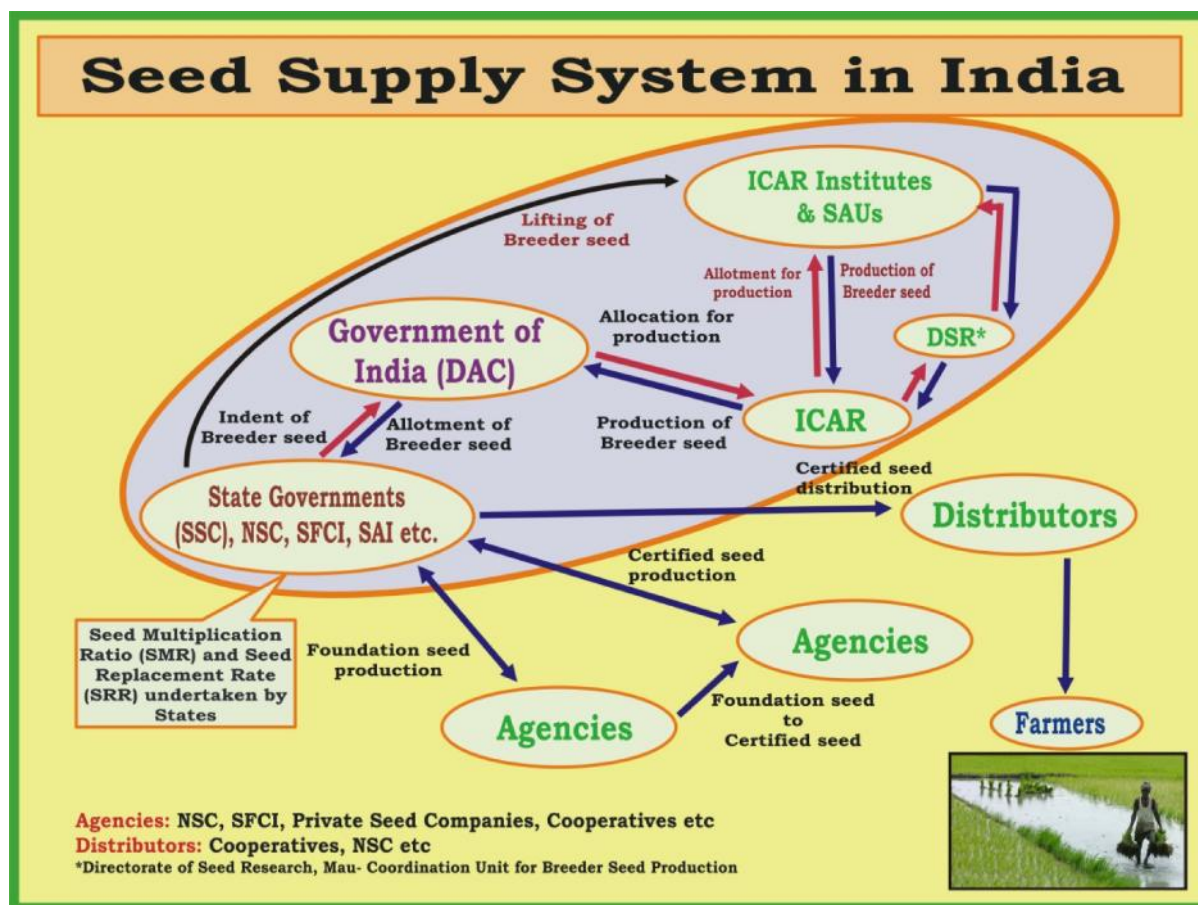
*Striving for prosperity of farmers is  
A National Initiative for information on quality seeds*

The screenshot shows the SeedNet India Portal website. At the top, there is a navigation bar with links: Central Sub-Committee Minutes, Inauguration, Search, Conferences, Tenders, Feedback, Search Variety, Contact, Circulars, Accolades. Below this is a banner for 'Seednet India Portal' with the tagline 'Striving for prosperity of farmers' and a sub-banner for 'National Initiative for information on quality seeds NEW of Rabi Crops d new alternative varieties- Aug 12'. The main content area is divided into several sections: 'Home', 'Breeder Seeds', 'Foundation Seeds', and 'Certified Seeds'. Each section contains sub-links and news items. For example, under 'Breeder Seeds', there are links for 'Recommended System', 'Research Institutes', 'Producing Agencies', and 'Allocation: Potato Allocation for Rabi 2014-15'. Under 'Foundation Seeds', there are links for 'Requirement & Availability' and 'Agencies/Institutes'. Under 'Certified Seeds', there are links for 'Requirement & Availability For Kharif-2013' and 'Agencies/Institutes'. There are also sections for 'Legal & Admin Framework', 'Organisations', and 'Quick Links'. The footer includes 'Best viewed in 1024 X 768 pixel screen resolution' and 'Designed and Developed by Agricultural Informatics Division, National Informatics Centre'.

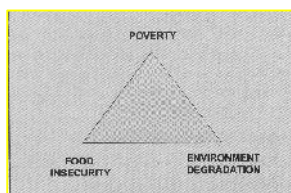
Strengthening of Seed Informatics & Communication (SeedNet) is a seed specific portal. It involves developing necessary databases / applications so that different agencies could provide and update requisite information. Various users, particularly farmers, will be able to access the information from a single source. It would provide smooth functioning among all the various constituent agencies for achieving qualitatively better information management for the complete seed sector.

Information about the requirement, production and availability of Quality Seeds at different State Agricultural Universities and Breeder seed producers is available. The SeedNet portal also provides all government orders, Acts / Control Orders, Rules, Quality Control measures, extension services, Export and Import of seeds and Programmes and schemes of the Centre etc. The salient features of over 3000 varieties, notified/released are available online from 1966 till 2007. The complete **Seed Supply Chain** from Indent to Allocation has been made online.

(Courtesy: NIC, DAIC project, New Delhi).



**Major concerns:** Poverty, food security and environment degradation are 3 major concerns to us



**Future Needs and Projections:** A target of 230 million tonnes of food grains have been protected by 2000 AD. Obviously, this target could be achieved only if efficient agriculture technology is available. The arable land being limited, it is not possible to bring fresh areas under cultivation. Therefore, the only way to achieve agricultural growth is through increased productivity, which is possible with the widespread use of quality seeds. Without a strong support from seed industry, it is not possible to conceive the nation.

To guaranty all this seed programme viz., production, supply, distribution and maintenance of seed quality and seed health in the world wide seed trade it is absolutely necessary to have well defined and planning coupled with unique knowledge transfer systems at all levels for updating the technical skill on all the seed related aspects for the benefit of officials, scientists, seed producers, farmers and stakeholders.

Acknowledgements and documents referred: The contributors of National training on seed quality regulation & seed health testing during 2009-10; Varietal purity-Testing specified traits and Seed Congress organized by NSRTC, Varanasi during 2008-09 & 2009-10.

## Maintenance Breeding In Maize, Rice and Cotton

### Importance of maintenance breeding:

Seed is one of the most vital and critical input for increasing agricultural production. All other inputs will go as waste if the seed is not good. Seed acts as a catalyst in agricultural production.

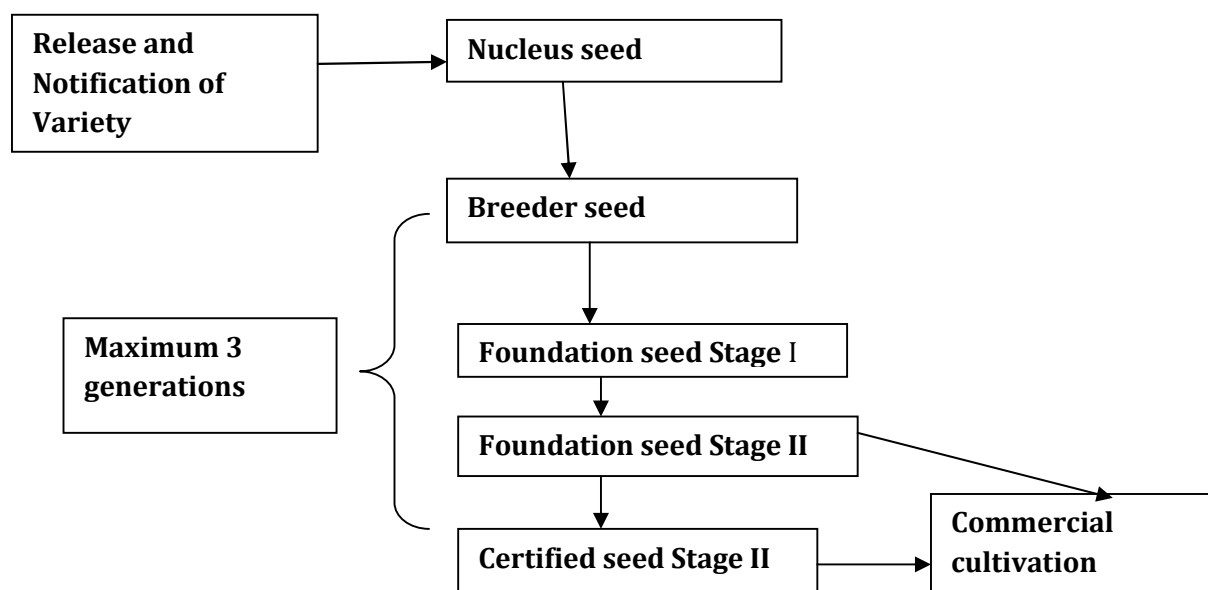
The important factors, which affect the genetic purity of varieties, are as follows.

- Developmental variations
- Mechanical mixtures
- Mutations
- Out-crossing
- Minor genetic variations
- Selective influence of diseases
- The technique used by the plant breeder

### Natural out-crossing depends upon

- The prevailing breeding system of the species
- Isolation distance
- Varietals mass
- Pollination agents

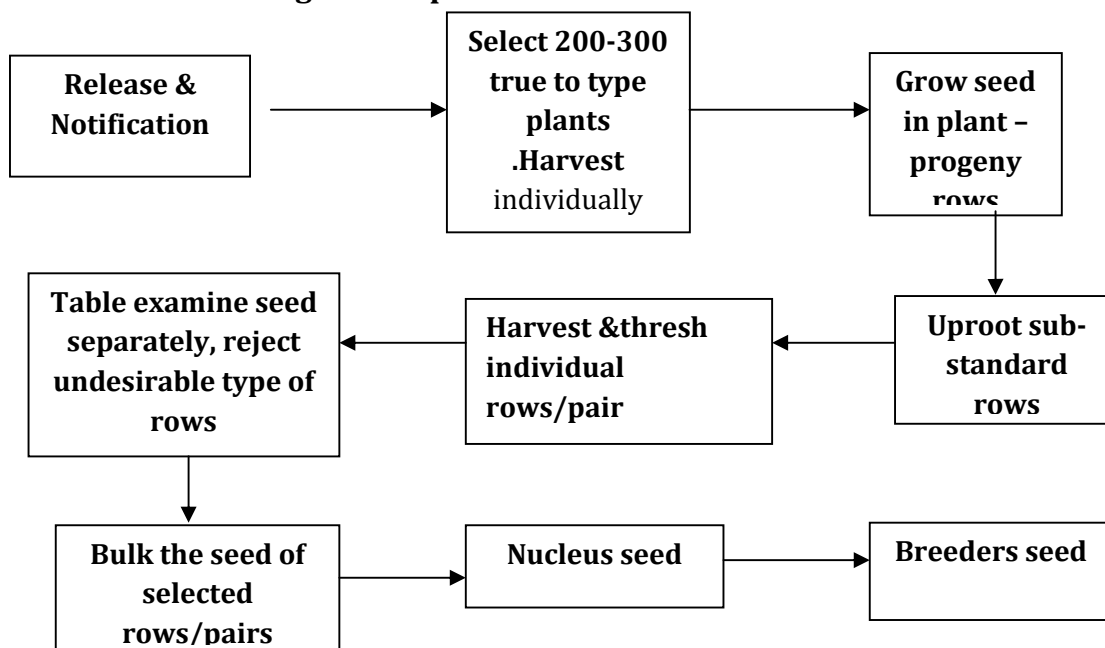
### Generation / Steps in Seed Multiplication Programme



### Classes of seed:

1. Nucleus seed
2. Breeder seed
3. Foundation seed
4. Certified seed

### Schematic diagram for production of nucleus seed



In hybrid crops maintenance of male sterile line (A), maintainer (B) and restorer line (R). Parental lines are multiplied / maintained separately in isolated plots by plant or ear to row method. Nucleus seed of female line (A) is maintained by undertaking mass selection in B line. Ear to row progenies of B line are grown in isolation adjacent to A line. The off-type progenies of A and B lines are removed. Seed parent rows are hand pollinated by collecting pollen from desirable B line progenies. Individual plants of B line are – selfed. Similarly the parental stocks of R lines are maintained in isolation.

**Note:** If needed the nucleus seed may have one more multiplication. It is desirable if the breeder grow head to progeny row for purification / maintenance of the variety.

### Breeder involved in nucleus / breeder seed multiplication should know

- Breeding behaviour and the impact of environmental conditions on the crop particularly.
- The diagnostic characteristics of the variety / parental lines dealing with.
- Specific requirement of the crop / variety / parental lines like isolation, land requirement, disease infection etc.

### Monitoring of breeder seed

The breeder seed is not certified however, for maintenance of quality, it is the responsibility of the monitoring system to maintain the quality of the breeder seed to a great extent.

### The constitution of Monitoring Teams include:

- Crop breeder / Representative of Crop Coordinator
- Producing breeder
- Representative of State Seed Certification Agency
- Representative of National Seeds Corporation

### Important precautions

1. Land requirements
2. Isolation distance
3. Use of machines
4. Line sowing
5. Roguing
6. Harvesting, threshing and processing
7. Quality assessment
8. Packaging and labeling

|   |                |
|---|----------------|
| Crop / Variety                          | Label No ----- |
| Lot No.                                 |                |
| Date of Test                            |                |
| Physical Purity (Min)                   |                |
| Genetic purity (Min)                    |                |
| Inert Matter (Max.)                     |                |
| Treated with                            |                |
| Germination (%)                         |                |
| Valid up to                             |                |
| Producing Institute<br>(Name & Address) |                |
| Weight                                  |                |

### 9. Storage

### 10. Grow Out Test

#### 10.1. Sampling:

1,000 g – for maize, cotton, groundnut, soybean and species of other genera with seeds of similar size.

500 g – for sorghum, wheat, paddy and species of other genera with seeds of similar size.

250 g – Beta and species of other genera with seeds of similar size.

100 g – for Bajra, jute and species of all other genera



**Specifications for different crops:**

| Sr. No. | Crop                             | Row length (m) | Distance (cm)  |              |               | No. of replications |
|---------|----------------------------------|----------------|----------------|--------------|---------------|---------------------|
|         |                                  |                | Between plants | Between rows | Between plots |                     |
| 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:                           |                |                |              |               |                     |
|         | a) very early to medium          | 6              | 15             | 20           | 45            | 2                   |
|         | b) late and very late            | 6              | 25             | 30           | 60            | 2                   |
| 7.      | Pearl millet                     | 6              | 10             | 60           | 90            | 2                   |
| 8.      | Sorghum                          | 6              | 10             | 45           | 60            | 2                   |

| Maximum permissible offtypes (%) | 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  |

**Maintenance Breeding in Maize ( *Zea mays* )*****Maintenance breeding serves the following purposes:***

It helps in purification and maintenance of a variety and consequent production of nucleus seed.

It extends the useful life of a variety.

It helps in meeting the uniformity criteria in DUS testing.

The quality nucleus seed reduces the amount of rouging in breeder seed

The concept of variety maintenance is related to genetic purity

The variety, which has been released and notified, must satisfy certain criteria of Distinctness, Uniformity and Stability (DUS) and value for cultivation and use (VCU). These parameters must be maintained over the generations.

**Based on the Characteristics of endosperm maize can be classified as:**

**Pop corn:** Small smooth kernel with hard endosperm.

**Flour type:** Large Smooth kernels with floury endosperm.

**Flint corn:** Large smooth kernels, mainly hard endosperm but often with a small floury centre

**Dent type:** Large kernel with a center core of floury endosperm, which on drying shrinks more than surrounding hard tissue denting the kernel.

**Sweet corn:** Carbohydrates stored largely as sugars, kernels wrinkle and turn translucent when dry.

**Floral biology and pollination**

Monoecious Plants

Tassel (♂ male flowers)

Cob (♀ female flowers)

Protandrous crop

Maize produces large pollen grains (90-125 μ x 85 μ )

Pollen volume of weight is approximately 700 x 10<sup>-9</sup> cc and weight 250 x 10<sup>-9</sup> g respectively.

Pollination through wind

Tassel shed the pollen for 2–14 day (depending on genotype and environment)

Each plant is capable of producing 9000 – 50000 pollen per kernel set

Anthers with a minimum overlap results in approximately 5% self fertilization.

Silks are receptive throughout its length and remain receptive up to 10 days

After fertilization the silks stop elongation and desiccate rapidly

Till it is get pollinated the silk go on increasing its length

**Important diagnostic characteristics**

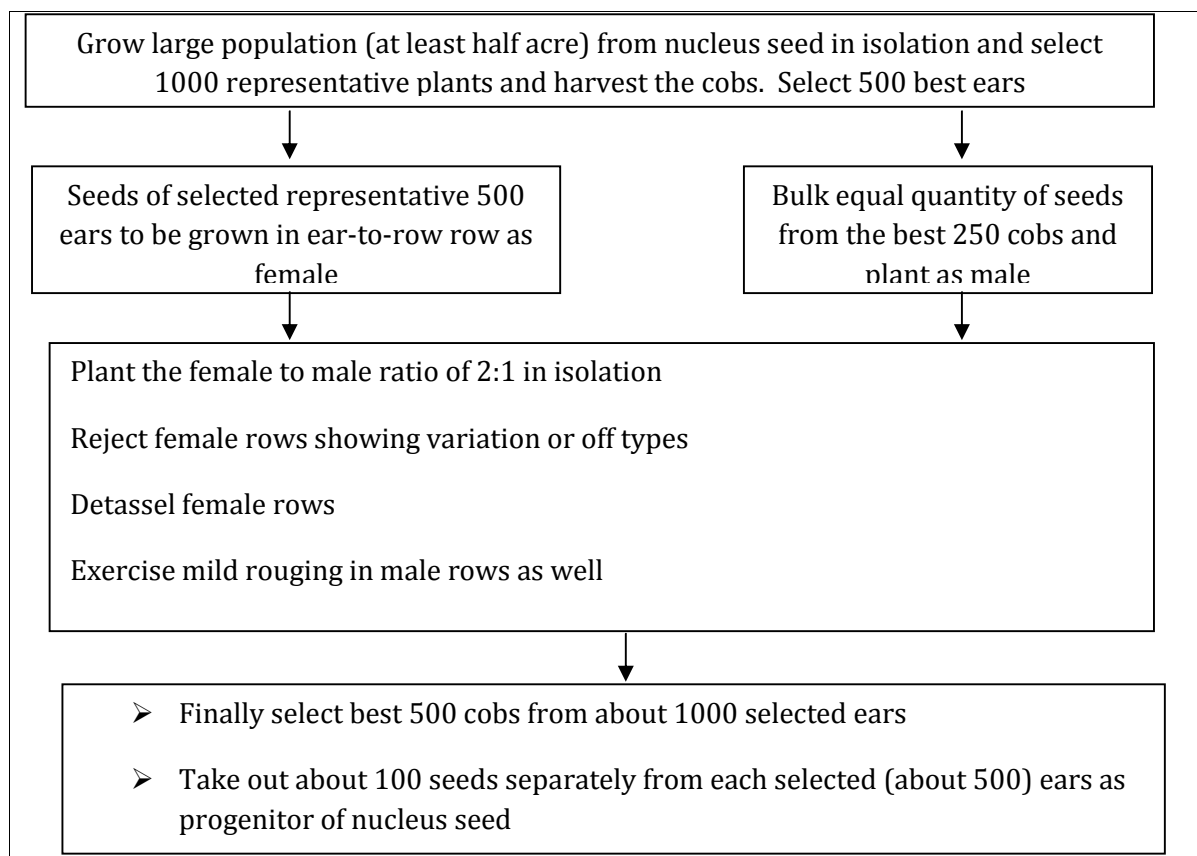
| S. No. | Characteristics                              | States                  | Stage of observation |
|--------|--|-------------------------|----------------------|
| 1      | Anthocyanin colouration of sheath            | Absent / weak / strong  | Seedling             |
| 2      | Anthocyanin colouration of brace roots       | Absent / present        | Reproductive         |
| 3      | Width of leaf blade                          | Narrow / medium / broad | Vegetative           |
| 4      | Angle between main axis and lateral branches | Small / medium / large  | Reproductive         |
| 5      | Number of primary and                        | Absent / few / many     | Vegetative /         |

|     |  |  |                         |
|-----|--|--|-------------------------|
|     | lateral branches                         |  | reproductive            |
| 6.  | Plant Height                             | Short/medium/long  | Vegetative/reproductive |
| 7.  | Time of silk emergence                   | Early/medium /late   | Silk emergence          |
| 8.  | Anthocyanin colouration at base of glume | Absent / Present   | Reproductive            |
| 9.  | Time of anthesis                         | Very early/ early / medium / late / v.late                                     | Reproductive            |
| 10  | Anthocyanin colouration of anthers       | Absent / Present   | Silk emergence          |
| 11. | Density of spikelets                     | Lax / medium / dense   | Reproductive            |
| 12. | Anthocyanin colouration of silk          | Absent / Present   | Silk emergence          |
| 13. | Length of peduncle                       | Short / medium / dense   | Reproductive            |
| 14. | Anthocyanin colouration of glumes of cob | White / light purple/ dark purple  | Reproductive            |
| 15. | Ear diameter                             | Small / medium /large  | Reproductive            |
| 16. | Ear shape                                | Conical / cylindrical  | Reproductive            |
| 17. | Number of rows of grain                  | Few / medium / many  | After harvest           |
| 18. | Type of grains                           | Flint/ semi dant / semi flint / dentpop / waxy / opaque / opaque tinge / sweet | After harvest           |
| 19. | Row arrangement of grains                | Straight / spiral / irregular  | After harvest           |
| 20. | Grain type                               | Shrunken / round / indented / pointed  | After harvest           |
| 21. | Grain size                               | Small / medium / bold  | After harvest           |

?

**Fig 1.**

**as such**



### Maintenance of Composites / OPV's

- OPV refers, which share a common gene pool
- Synthetics – derived through interbreeding of lines with good general combining ability
- Composite – interbred populations of advance generations of promising genotype without knowledge of general combining ability.
- Seed production is simpler and relatively inexpensive
- OPV's are particularly suitable for tribal and hilly regions
- Only off-types plants should be removed
- Mild selection during seed production and multiplication are inevitable
- Varietal maintenance should be done in isolation following half sib method.

### =Nucleus Seed of OPV

- From the variety 50–100 seed are to be bulked from each representative cob from the representative plants to form nucleus seed.
- 5000 – 10000 seeds are normally sufficient to represent OPV and provide nucleus seed.

### Maintenance and production of nucleus and breeder seed of inbred lines

- Inbred lines are derived through rigorous selfing/ and or sib mating (7 – 8 cycles)
- Inbred line maintenance is maintain the performance, appearance (physical and genetic purity).
- Proper isolation

- Rigorous elimination of off-types
- Care in pollination or procedures (selfing or sibbing)
- Maintain pedigree records.

### **Maintenance Breeding of RICE ( *Oryza sativa.L* )**

Botanical Description:

Habitat: Tropical and Sub tropical hydrophytic plant, grown as semi-aquatic crop

Habit: Annual herb

Root system: Adventitious

Shoot system:

Stem: Aerial, erect, cylindrical, hollow, nodes and internodes. It is known as culm.

Branching through tillers, floating rice long inter nodes.

Leaf: Semi-amp lexical, sheathing leaf base, ligulate (membranous), a ariculates (hairs fringed which clasp the stem), flag leaf or boot leaf.

Floral biology:

Terminal panicle, single flowered spikelets, each spikelet consists of two short glumes, a lemma and a palea, enclosing androecium and gynoecium. lemma 5nerved ,palea 3-nerved, two lodicules which are thick, fleshy, and hygroscopic, stamens 6 arranged in two whorls of three each, monocarpellary, unilocular ,superior ovary with single ovule on basal placentation.

#### **Anthesis and Pollination:**

- Panicle takes two to three days for complete emergence from the boot leaf. Maximum flowering 2-3 days after the emergence.
- Flowering top down wards, branches upwards
- On main axis there will be 8-10 nodes giving rise to 8-10 primary branches on which secondary and tertiary braches arises
- The swelling of lodicules due to hygroscopic and rapid elongation of the filaments pushes lemma and palea to open it.
- In general most of the spikelets open between 8.00am to 12.00 noon
- Blooming varies with the variety and locality, it is delayed during cloudy days
- It takes 4-10 days for complete blooming
- Rice is protandrous crop, self pollination is the rule
- In normal varieties the spikelet remain open for half an hour to one hour, where as CMS line one and half to two hours
- The spikelets close after pollination and do not open again
- In hybrid rice out crossing ranges 20-40%
- Stigma receptive for 3 days, pollen viable only 5 minutes. Self pollination is the rule

**Important Diagnostic Characters in Rice:**

| S.No. | Charectoristic  | States   | Stage of observation |
|-------|---|--|----------------------|
| 1.    | Coleoptiles colour  | Colourless /green/purple   | Seedling             |
| 2     | Leaf sheath colour  | Absent/present   | Vegetative           |
| 3.    | Leaf blade colour   | Pale green /green/dark green   | Vegetative           |
| 4.    | Purple tip / purple margin                                  | Purple blotch / purple mixed with green / purple   | Vegetative           |
| 5.    | Leaf pubescence   | Glabrous / hairy / velvety   | Vegetative           |
| 6.    | Leaf shape  | Acute / truncate /split  | Vegetative           |
| 7.    | Ligule colour   | White / light purple / purple  | Vegetative           |
| 8.    | Flag leaf angle   | Erect / semi erect / horizontal  | Vegetative           |
| 9.    | Panicle exertion  | Partly exerted / exerted / Well exerted  | Reproductive         |
| 10.   | Panicle Type  | Compact / Intermediate / open  | Reproductive         |
| 11.   | Panicle length  | Short / medium / long  | Reproductive         |
| 12.   | Lemma anthocyanin   | Absent or very weak / weak /   | Vegetative           |
| 13.   | Colouration of keel   | medium / strong / very strong  | Vegetative           |
| 14.   | Lemma anthocyanin vegetative colouration of area below apex | Absent / weak / medium / strong v.strong   | Vegetative           |
| 15.   | Apiculous colour  | Green / purple / red   | Reproductive         |
| 16.   | Awning  | Absent / short and partly awned / short and fully awned / long and partly awned / long and fully awned | Reproductive         |
| 17.   | Awn colour  | Yellowish white / brown / reddish brown / light red / red / light purple / purple / black              | Reproductive         |
| 18.   | Stigma colour   | White / light green / yellow / light purple / purple   | Reproductive         |
| 19.   | Pollen fertility(in male sterile line plots)                | v Sterile (shriveled white anthers)/ semi sterile (partly sterile)                                     | Flowering            |

|     |  |   |           |
|-----|--|---|-----------|
| 20. | Spikelet fertility(in male sterile line plots) | Fertile (spikelets filled)/ sterile Maturity (spikelets empty)/ semi- sterile (Partial sterile)         | Flowering |
| 21. | Kernel colour                                  | White / light brown / variegated brown / dark brown / light red / red / variegated purple / dark purple | Maturity  |
| 22. | Grain size                                     | Round / short slender / short bold / medium slender / medium bold / long slender / long bold            | Maturity  |
| 23. | Abdominal white                                | present / absent occasionally present (White belly)   | Maturity  |

### Maintenance Breeding of (A B & R ) Parental Lines

Rice hybrids are developed by using CMS system by involving three lines

- I. A line or CMS line (male sterile)
- II. B line or maintainer line (male fertile)
- III. R line or restorer line (male fertile)

The success of hybrid seed production programme depends on the high genetic and physiological purity of parental lines, also on efficient and economic seed production for every 1% decrease in purity of hybrid seed, yield of hybrid rice decreases about 100 kg as is reported in China

- Multiplication of A line which involves crossing of AxB lines
- Purification of parental lines is the most important pre-requisite to ensure the purity of hybrids
- Parental line need to be purified under the control of plant breeder
- Knowledge on the typical characteristics of parental lines is essential

### Purification process involves four steps:

- I. Source nursery- Growing the source material
- II. Test cross nursery- Test crossing of selected lines
- III. Identification nursery- Evaluating the test crosses
- IV. Multiplication nursery – Multiplication of identified lines

#### 1. Source Nursery (Season- I)

- Large number of A,B and R lines are grown side by side for convenience
- 'B' line is sown 4-5 days after A line and R line is staggered depending on the flowering difference between A & R line
- The individual plants are observed and the off types are carefully removed

- At flowering 200-250 typical CMS plants which are completely sterile and typical maintainer and restorer lines are selected base on their key distinguishing characteristics

## 2. Test Cross Nursery (Season- II)

- Approximately 200-250 paired crosses are made between selected plants of CMS line with those of maintainer lines /plant
- Two or three panicles of the same CMS plant in A line are used for crossing with a selected B line plant, and another 2-3 panicles of the same plant for crossing with the selected 'R' line plants.
- At least 100-150 seeds are produced for each pair of A x B cross, while 30-40seeds would be enough for the A x R cross
- Label the crossed panicles of A x B as  $A_1 \times B_1, A_2 \times B_2 \dots A_n \times B_n$  and similarly the A x R crosses are labeled as  $A_1 \times R_1, A_2 \times R_2 \dots A_n \times R_n$  .The seeds have to be harvested and threshed with due care.

## 3. Identification nursery (Season- III)

**The main purpose of this nursery is to identify the progenies that are not uniform or true to type so that it can be eliminated**

- a. A x B crosses, plants are selected for complete male sterility
- b. A x R for higher spikelet fertility and uniformity

**The steps involved are as follows:**

- The purpose of identification nursery is to identify those progenies, which are not uniform and true to type.
- 10-20 seeds from each of the A x B crosses and all the seeds of A x R crosses are used for raising the nursery.
- The paired crosses of A x B and A x R are planted in the main field without isolation
- Observe the progenies of A x B crosses for stable male sterility, true to type and uniformity
- The off- types, partial fertile plants in A x B crosses and those with poor restoration in A x R crosses are identified.
- It is very important to identify the plants which deviate from normal standard characteristics in identification nursery so as to reject them in multiplication nursery.

## 4. Multiplication nursery (Season III)

- After sowing a part of A x B crosses seed in identification nursery, remaining seed is sown 21 days later along with corresponding 'B' lines. This material is planted in isolated plots of more than 500 m.
- Based on the observations made in A x B crosses in the identification nursery, deviants are removed before flowering along with their corresponding 'B' lines in multiplication nursery.
- Remaining A x B pairs is allowed to cross – pollinate.
- 'B' lines are harvested first, threshed separately and bulked. This forms the source seed for nucleus seed production of 'B' lines.



- Then 'A' lines are harvested and threshed separately. This forms the source for nucleus seed production of 'A' line.

## 5. Restorer line multiplication

- The restorer lines are grown as panicle to row progenies in a separate block, 21 days after transplanting of A x R progenies in identification nursery.
- Those lines which are found to be deviant in A x R progeny as observed in identification nursery are removed before flowering.
- After assessing spikelet fertility of the corresponding A x R progenies, the seeds of the corresponding 'R' lines with good restoration are bulked as source seed for nucleus seed production of 'R' line.

## Nucleus seed production of 'A' line

- Isolation of 500 m is necessary for nucleus seed production of 'A' line.
- Seed rate: 15 kg ha<sup>-1</sup> for 'A' line, 5 kg ha<sup>-1</sup> for 'B' line
- Staggered sowing of 'B' line is necessary. Half the quantity of 'B' line is sown 3 days after the sowing of 'A' line and the remaining half of the 'B' line is sown 5 days after the sowing of 'A' line.
- While transplanting, the recommended spacing of A, B line and row ratio should be followed.
- While transplanting, the following steps may be followed :

|                               |   |       |
|-------------------------------|---|-------|
| Spacing between A and B lines | : | 30 cm |
| Spacing between A lines       | : | 15 cm |
| Spacing between two 'B' lines | : | 30 cm |
| Plant to plant spacing        | : | 15 cm |
| Row-ratio of (A : B)          | : | 6:2   |
| Plant single seedling /hill.  |   |       |

Row direction should be perpendicular to the wind direction to facilitate higher out crossing. The following points may be borne in mind while taking up nucleus seed production of A – line:

1. Apply GA<sub>3</sub> (60 g ha<sup>-1</sup>) mixed in 500 liters of water to enhance panicle exertion and seed set, GA<sub>3</sub> is sprayed at 5-10% flowering on two consecutive days with 40% on the first day and 60 % on the second day.
2. Off – types i.e., pollen shedders in 'A' line and any other plants that do not conform to the characteristics of A line are removed. Rouging is done at all critical stages such as vegetative, flowering and maturity stage.
3. Supplementary pollination through rope pulling or rod shaking is done to enhance the cross-pollination.
4. Monitoring by competent personal is essential to produce the genetically pure seed.
5. At the time of harvest, 'B' line is harvested first and then only 'A' line.

6. Seed is cleaned and dried to 10-12% moisture content and treated before storing in cool and dry place.

### ***Nucleus seed production of B and R line***

- It is similar to the nucleus seed production of inbred varieties. Initially seed of purified B and R lines is used to produce nucleus seed and subsequently single plants are selected for further use.

### **Breeder seed production of parental lines:**

Breeder seed production of A line

It is similar to the nucleus seed production ( A X B )as described earlier, except row ratio which may be either 2:8 or 2:10.

Breeder seed production of B and R lines

The breeder seed production of B and R lines is produced from the nucleus seed as per the method prescribed for the varieties.

### **Maintenance Breeding of Cotton (*Gossypium spp*)**

#### **Botanical Description:**

Habit: Cultivated mesophyte

Habit: Annual herb, some are perennial shrubs

Root system: Normal tap root system which is very deep

Shoot system:

Stem: Stem is aerial, erect, solid, cylindrical with black nectar glands, woody stem at base, at vegetative region monopodial and at flowering region sympodial branching.

Leaf; Stipulate, stipules are free, petiolate, simple one or penta lobed. palmately reticulate venation, alternate phyllotaxy.

#### **Floral Biology:**

**Inflorescence:** Extra axillary terminal solitary born on sympodial branches

Flower: Bracteate, bracteolate, bracteoles are three usually foliar, persistent, toothed, bisexual, hypogynous, actinomorphic, inner side of the bract a small gland is present di and hetero chlamydeous

Calyx; Sepals-5, gamosepalous forming a truncate cup shortly five toothed with persistent nature.

**Corolla:** Petals are 5, polypetalous, contorted (twisted) in some varieties red colour eye spot is present at the base on the inner surface of the petals, yellow color.

Androecium: Stamens are numerous, monoadelphus stamina column unites with base of petals, epipetalous anthers are monothecous, reniform or kidney shaped extrose and basifixed.

**Gynoecium:** Pentacarpellary, Syncarpous, superior ovary penta locular with many ovules on axile placentation, style is terminal, passing through the stamina tube, stigma bifid.

**Fruit:** Loculicidal capsule called as boll, the locule is called lock. Aborted locules called motes. Long hairs called lint. Short hairs are fuzz. The lint is cotton of commerce. American cottons open earlier than the Asiatic cotton. Asiatic cotton opens between 8 to 10 am. Temperature effects the flower opening.

### Important distinguishing morphological characters in cotton

| <b>S. No.</b> | <b>Characteristics</b>           | <b>States</b>  | <b><u>Stage of observation</u></b>     |
|---------------|----------------------------------|--|--|
| 1.            | Hypocotyle pigmentation          | Absent/present   | <b><u>Seedling</u></b>                 |
| 2.            | Stem pigmentation                | Absent/present   | <b><u>Vegetative/ reproductive</u></b> |
| 3.            | Stem hairyness                   | Absent/medium/strong   | <b><u>Vegetative/reproductive</u></b>  |
| 4.            | Plant height                     | Dwarf/medium/tall  | <b><u>Vegetative/reproductive</u></b>  |
| 5.            | Leaf shape                       | Palmate(normal)/digitate(okra)/semi-digitate(semi-okra)/lanceolate(super okra) | <b><u>Vegetative/flowering</u></b>     |
| 6.            | Leaf size                        | Small/medium/large   | <b><u>Vegetative/flowering</u></b>     |
| 7.            | Leaf colour                      | Light green/green/light green/dark red   | <b><u>Vegetative/flowering</u></b>     |
| 8.            | Leaf pubescence                  | Absent/medium /strong  | <b><u>Vegetative/flowering</u></b>     |
| 9.            | Leaf nectories                   | Absent/present   | <b><u>Vegetative/flowering</u></b>     |
| 10.           | Leaf petiole pigmentation        | Absent/present   | <b><u>Vegetative/flowering</u></b>     |
| 11.           | Bract type                       | Normal/frego   | <b><u>Vegetative/flowering</u></b>     |
| 12.           | Sepal pigmentation               | Absent/present   | <b><u>Flowering</u></b>                |
| 13.           | Petal colour                     | White/cream/yellow/pink/red/bicolour   | <b><u>Flowering</u></b>                |
| 14.           | Petal spotting                   | Absent/present   | <b><u>Flowering</u></b>                |
| 15.           | Position of stigma               | Embedded/exerted   | <b><u>Flowering</u></b>                |
| 16.           | Anther colour                    | White/cream/yellow/purple  | <b><u>Flowering</u></b>                |
| 17.           | Boll size                        | Small/medium/large   | <b><u>First boll bursting</u></b>      |
| 18.           | Boll shape(longitudinal section) | Rounded/elliptic/ovate   | <b><u>First boll bursting</u></b>      |
| 19.           | Boll tip prominence              | Blunt/pointed  | <b><u>First boll bursting</u></b>      |
| 20.           | Boll surface                     | Smooth/pitted  | <b><u>First boll bursting</u></b>      |
| 21.           | Boll opening                     | Close/semi-open/open   | <b><u>First picking</u></b>            |
| 22.           | Fibre length                     | Very   | <b><u>First picking</u></b>            |

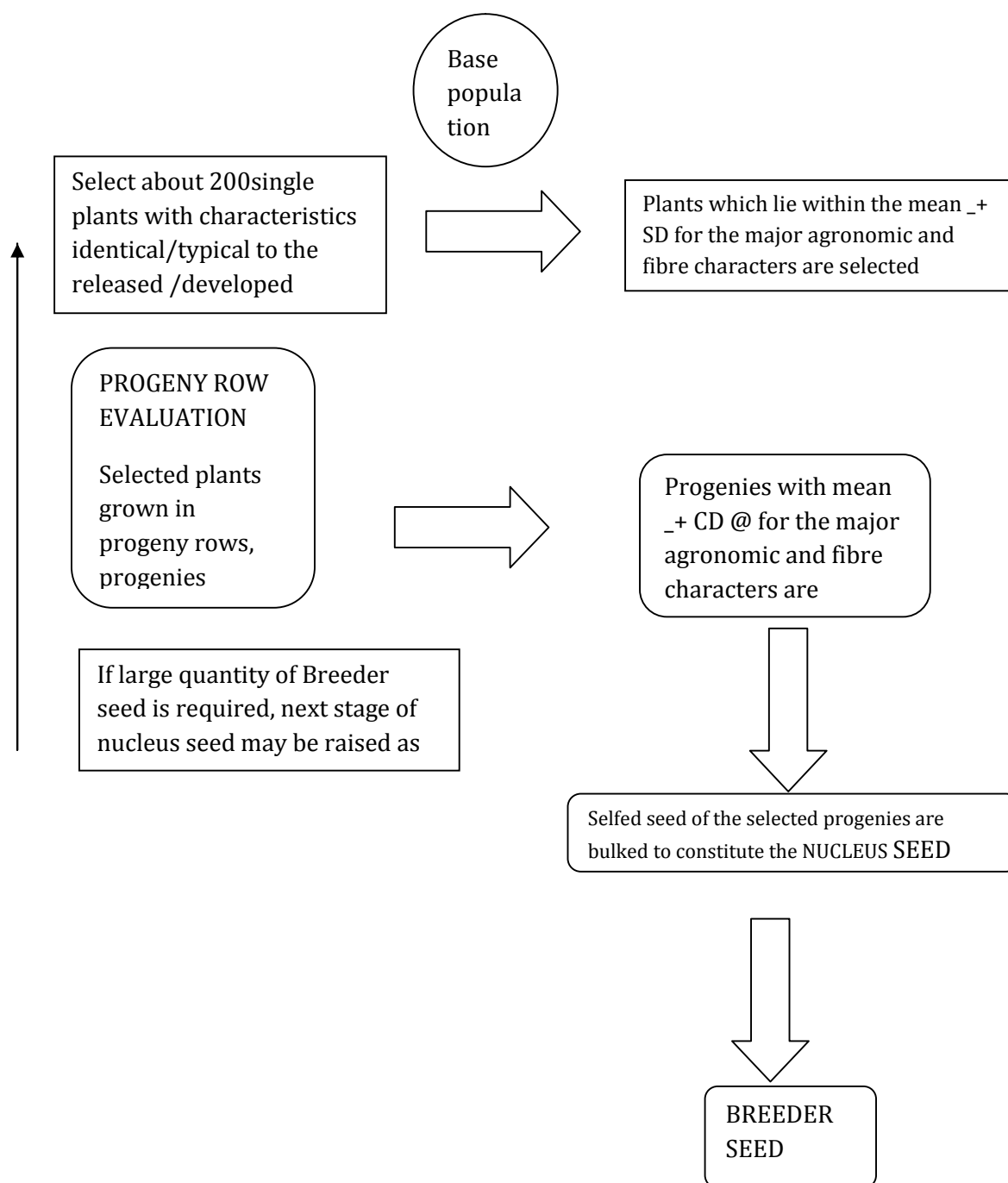
|     |                 |   |                                |
|-----|-----------------|---|--------------------------------|
|     |                 | short/short/medium/long/extra long                |                                |
| 23. | Fuzz colour     | White/grey/brown/green/                           | <b><u>Harvest maturity</u></b> |
| 24. | Fibre colour    | White/off white/brown/green                       | <b><u>Harvest maturity</u></b> |
| 25. | Fibre strength  | Weak/ medium/strong                               | <b><u>Harvest maturity</u></b> |
| 26. | Ginning percent | Low/(<31)medium(31-35)/high(36-40)/very high(.40) | <b><u>After ginning</u></b>    |
| 27. | Density of fuzz | Naked/semi fuzzy/fuzzy                            | <b><u>Harvest maturity</u></b> |

### **Nucleus and Breeder seed production of Varieties**

- Select ( minimum 200 plants) from the base population or source
- Compare the morphological traits with the variety released/developed
- Selected plants are selfed
- Mean and S.D are to be worked out, the plants that lie within the mean  $\pm$ SD for all the characters are selected
- The selected plants subjected to fibre quality. 2.5%span length, micronaire uniformity ratio
- The selfed seeds from the selected plants are grown in a R.B.D
- Normally two rows of two replications per progeny are grown
- Nucleus seed production should be taken under most favourable climatic conditions where the variety is most adopted
- Grow progeny rows in compact field with proper isolation
- Reject deviant plant ,diseased plant progenies
- Selfing is to be done to maintain the purity
- Progenies that fall within the mean  $\pm$  CD @ 5%are selected
- Equal quantity of selfed seeds of selfed progenies is bulked to constitute the nucleus seed
- If large quantities of BSP is needed next stage of progeny bulk seed may be taken as nucleus seed stage II

### **BSP:**

BSP should be taken where the variety is most adopted during the best growing season. Isolation distance 50 m , breeder should critically monitor any deviant plant it should be rouged out immediately



#### Breeder seed production of varieties:

- The nucleus seed is utilized for BS
- BSP should be taken areas of variety adaptation
- Isolation distance 50 m
- Plot is observed the breeder for morphological characters
- Deviant plant(s) should be removed

#### Nucleus/breeder seed production of parental lines of conventional hybrids:

Parental lines of hybrids are produced, similar way described for varieties  
Male and female parents are maintained separately in isolation  
Selected lines are tested for SCA and heterosis for yield and fibre quality traits are  
utilized for further multiplication and hybrid seed production

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## Seed Production in Maize (OPV/Hybrids): Field and Seed Standards

### Introduction

Maize (*Zea mays* L.) is the multi utility crop with wider adaptability and highest genetic yield potential among the cereals. It is an important cereal crop in world after wheat and rice. Maize is unique among the cereals on account of various features which make it as one of the top three cereals and its amenability to diverse uses is unparalleled. Ranging from pharmaceuticals to many other industrial uses like biofuel, besides food, feed and fodder purpose, diverse corns find their place. There are many types of maize based on the grain composition such as dent, flint, pop, pod, waxy and floury maize. Maize occupies an important place in world agriculture. It is grown in more than 150 countries. The major producing countries are USA, China, Brazil, Argentina, Mexico, India. At global level, India ranks 4<sup>th</sup> in area and 6<sup>th</sup> in production of maize. In India as per the latest report, maize area, production and productivity is 8.71 mha, 22.23mt and 2.55 t/ha, respectively during 2012-13 (DAC, 2012). Maize productivity is relatively higher in the states like Karnataka, Andhra Pradesh, Bihar, Punjab and Himachal Pradesh. The productivity of Andhra Pradesh is highest in India. Utility pattern of maize in India are as source of human food 25%, as animal feed 12%, in poultry feed 49%, in starch industry 12%, brewery 1% and as seed 1%.

Maize is widely cultivated throughout the world, and a greater weight of maize is produced each year than any other grain. In Asia maize is widely cultivated in many countries and among the ASEAN countries leading producer of maize is Indonesia followed by Philippines, Thailand and Myanmar (Table-1). Production can be significantly could increased in rest of the ASEAN countries through mutual benefit sharing programmes. For this, sharing of expertise in the field of seed production in maize have huge potential.

**Table-1 Area, production and productivity of world, Asia, India and ASEAN countries during 2012. (source FAOSTAT, 2012)**

| Country                    | Yield (Kg/Ha) | Production (M. tons) | Area (in Ha) |
|----------------------------|---------------|----------------------|--------------|
| <b>I. World</b>            | 4944          | 875.10               | 176.99       |
| <b>II. Asia</b>            | 5007          | 287.92               | 57.49        |
| <b>III. India</b>          | 2507          | 21.06                | 8.40         |
| <b>IV. ASEAN Countries</b> |               |                      |              |
| 1. Indonesia               | 4893          | 19.38                | 3.95         |
| 2. Malaysia                | 5200          | 0.05                 | 0.01         |
| 3. Philippines             | 2856          | 7.41                 | 2.59         |
| 4. Singapore               | --            | --                   | --           |
| 5. Thailand                | 4457          | 4.81                 | 1.08         |

|             |      |      |      |
|-------------|------|------|------|
| 6. Brunei   | --   | --   | --   |
| 7. Myanmar  | 3947 | 1.50 | 0.38 |
| 8. Cambodia | 2394 | 0.79 | 0.33 |
| 9. Laos     | 5719 | 1.13 | 0.20 |
| 10. Vietnam | 4295 | 4.80 | 1.12 |

### Maize breeding research in India

The focused research in SCH helped in addressing several issues of biotic and abiotic stresses *viz.*, lowering water table, rising temperature, etc. The success story of SCH in US Corn belt is well known. Its impact has been realized in China, Brazil, Canada and many other countries too. Even in USA with cultivation of Open Pollinated Varieties the productivity remained less than 2 tons/ha. And further, the results were not encouraging with the coverage of 100% area under double cross hybrids and the productivity was only 3.5 tons/ha over a period of 25 years (1936-1930). But with the adoption of SCH technology in 1960s USA productivity increased 3.5 tons/ha (1960) to 9.68 tons/ha (2008). The annual increase in productivity with 100% coverage under double cross hybrid was only 60kg/annum in 25 years and with single cross hybrid cultivation the productivity per annum is more than double in a period of 50 years. Parallel to USA in India the productivity remained less than 1 ton/ha for many decades continued. After shifting to SCH technology (2006-2008), in India has witnessed 30% increase in production and 27% increase in productivity within two years with the coverage of 20% area under SCH. There is also 15% annual increase in production and more than 12% increase in productivity. India became net importer to potential exporter. This is the visible impact of single cross hybrid technology. So maize breeding strategies in different period of time (Table-2) has been adopted in India as follows

**Table-2 maize breeding strategies in different period**

| Period       | Maize Breeding Strategies   |
|--------------|---|
| 1950-60      | Land races  |
| 1960-67      | Double cross (DC)   |
| 1967-71      | Composite   |
| 1971-89      | Composite and Double top cross (DTC) / DC                           |
| 1989-2000    | Single Cross Hybrid (SCH)/DC / Three Way cross (TWC) and composites |
| 2000-06      | SCH/TWC/DC and composites   |
| 2006-onwards | Single Cross Hybrids  |

### Reproductive biology:

#### Inflorescence

Maize is a monoecious plant i.e. male and female inflorescences are located at separate places on the same plant which cross pollination a general rule. However, five



per cent self – pollination is also recorded. The male flowers are borne in cluster (called tassel) on the top of the stem as a terminal panicle. The branches of the tassel are spirally arranged around the axis. The female flowers are borne inside the young cobs, wrapped under bracts, which arises from one of the nodes on the stem usually located about midway on the stalk.

### **Male Flower**

The spikelets are usually arranged in pairs one sessile and the other pedicellate (stalked). Each spikelet is enclosed by two glumes. There are two functional florets per spikelet. Each floret is enclosed between the lemma and palea and contains three stamens with linear and pendulous anthers, two small cup –shaped lodicules and rudimentary pistil (Weather 1955).

### **Female flower**

The female spikelets are densely packed in several vertical series on the thick and cylindrical rachis. Each spikelet is enclosed two membranous, broad and empty glumes. Lodicules are absent or very feebly developed. The spikelet has a lower barren (extremely reduced) and an upper fertile floret. Each floret enclosed between lemma and palea (Dutta 1971). The style is very long silky filament and in the cluster is known as silk.

Silk become receptive as soon as they emerge from the ear husk. Generally, silk grow upto 10-15 cm in length and can retain viability upto 7-10 days in want of effective pollination (Walden and Everett 1961). Best seed sets occurred with pollinations three to five days after first silk emergence, but pollination after eight days still gave 66 percent seed set compared with optimum (Hallaver and Sears 1966).

### **Anthesis**

At anthesis, just prior to pollen shedding, the lodicules swell to several times of their normal size and push the palea and lemma apart, facilitating the anthers exertion through filament elongation. Anthers open at the tip, forming pores through which the pollen mass is discharged in huge numbers in wind. Moisture stress and high temperature of about 35-40 degree centigrade may also cause tassel firing. It is estimated that a tassel produces 25000 pollen grains for each female gamete in a normal environment (Kiesselbach 1994). After release and dispersal from anthers, pollen grains retain viability for few minutes only.

### **Synchronization of flowering**

Split date plantings of seed parents refer to the planting of the female and male parents on different dates. This practice is employed to optimize the synchronization of pollen shed and silking of the two seed parents of different maturity "nick " or reach the flowering stage concurrently (Wych, 1988). Male parents are often planted on two dates to extend the pollen –shedding period by the inbred male. Plantings are timed so that peak pollen shed coincides with the maximum exposure of silks by the female

parent. Other methods utilization to alter flowering dates to bring parents of differing maturities together for timely nick include clipping of flaming to delay crop development, variable planting depths, and variable fertilizer rates. The methods are not used widely because they can reduce seed yields.

**In maize crop following kinds of varieties/hybrids are being commercially growing:**

- A. Open-pollinated varieties**
- B. Synthetic varieties**
- C. Composite**
- D. Hybrid**

#### **A. Open-pollinated varieties**

OPVs are made up of genotypes that are selected based on phenotypic appearance and bulked without testing progeny performance previously or performance in hybrid combinations. Subsequent maintenance of the variety should be by open pollination, in isolated field.

#### **B. Synthetic varieties**

A variety synthesized by crossing inter-se a number of genotypes selected for good combining ability in all possible combinations, with subsequent maintenance of the variety by open pollination is known as synthetic variety (Allard, 1960). The genotypes that are hybridized to produce a synthetic variety can be inbred lines, clones, mass selected population or various other materials. Synthetics derived from early generation inbred lines have given encouraging performance in maize crop.

Synthetic varieties in maize widely used due to the following advantages (Allard, 1960): The presence of more variability in synthetic varieties when compare to double cross hybrids might allow more adoptable to changeable growing conditions, pest and disease infestations. Cost of synthetic variety is lower than hybrid seed cost due to which small and marginal farmers can adopt the synthetic varieties than hybrids.

#### **C. Composite varieties**

The term composite variety refers to a germplasm composite which is commonly used to designate a broad group of materials mixed together in many different ways, and include breeding materials put together on the basis of desirable characters, such as yield potential, maturity, disease resistance etc., followed by random mating. It was in India that a population improvement methodology was outlined by Dhawan (1963) for developing commercial varieties named as composite.

#### **D. Hybrids**

Hybrid seed of maize may generally be produced from following different cross-combinations:

1. Single cross hybrid ( $A \times B$ )
2. Three-way cross hybrid  $(A \times B) \times C$
3. Double cross hybrid  $(A \times B) (C \times D)$
4. Double-top cross hybrid  $(A \times B) \times$  Composite variety

### **1. Single Cross Hybrid**

It is a product of the cross between two potential inbred lines ( $A \times B$ ). It is highly uniform and heterotic requires three isolations for seed production. However, seed cost is more since the seed yield is less.

### **2. Three Way Cross Hybrid**

The hybrid is produced by crossing the F<sub>1</sub> of the cross  $A, B$  with another potential inbred line  $C$ . This type of hybrid seed production requires five isolations. Generally, three-way cross hybrid is produced where three inbred lines, which combine well, are available but fourth suitable inbred is lacking, otherwise, double cross is more economical.

### **3. Double Cross Hybrid**

It is a product of four potential inbred lines. Product of  $(A \times B)$  is crossed between the F<sub>1</sub> of  $(C \times D)$ . It requires seven isolations for seed production and seed cost is less and it is less uniform.

### **4. Double top cross**

The first generation resulting from the controlled crossing of a certified single cross and a certified open-pollinated variety. That is, One single-cross and one open-pollinated variety (OPV) or composite variety, are involved in this system. A hybrid seed from single cross is taken as female parent and OPV as male parent.

Single crosses (between two inbreds) are the best with respect to the level of performance and uniformity and have a great merit in the commercial seed production. The genetic purity of inbred parents can be easily maintained and genetically true to type F<sub>1</sub> seed can be produced year after year. In conventional, hybrid seed production, one of the major problems encountered has been the lack of good vigorous inbred lines. All hybrids other than single cross hybrid have heterogeneous F<sub>1</sub> generation, and heterogeneity increases with the number of parent involved. The single crosses have greatest attractiveness and phenotypic appeal, uniformity in kernel type and suitability for combine harvesting. However, being uniform, they lack population buffering and possess only individual buffering, whereas three-way and double crosses have both population as well as individual buffering (Allard and Bradshaw 1964)

### **Maize hybrid seed production consists of three stages (Table-3)**

(Every stage of seed production is carried out in isolation)

**Table:3 three stages of hybrid seed production in maize**

| Stage of seed productions | Particulars   | Remarks  |
|---------------------------|---|--|
| 1. Breeder seed           | Parental lines are increased in limited area  | Parents should have genetic purity and certifying standards.   |
| 2. Foundation seed        | The seed obtained on male and female rows is called foundation seed   | Parents should have genetic purity and Certifying standards.   |
| 3. Certified seed         | Male and Female single crosses are generally sown in 2:4 ratio. The seed obtained on female parent is called certified Seed or Hybrid seed. | Detasseling should be attended in all female plants at proper time. Both single crosses (Male & Female) should posses genetic purity and certifying standards. |

**For a successful Hybrid seed production following pre -requisites are required**

- Male and female parental lines.
- Knowledge of flowering behaviour.
- Proven crossing techniques.

When commercial maize for 100000 ha has to be produced 0.2 ha area of breeder seed production and 10 ha of foundation seed production and nearly 667 ha of certified seed production is required. In Maize three different kinds of hybrids can be produced, that is the breeder seed is produced by the original breeder under his purview. While foundation seed production is taken up state Seeds Corporation, etc., and the certified seed production (F1 hybrid seed production) is usually done in the farmer's field (seed growers). Foundation and Certified seed production is done under the supervision of the Certification Agency.

**Agronomic practices followed during seed production in maize****Climatic Conditions**

Clear environment, with ample sunshine is the ideal place. However , very high or very low temperature during seed production period is harmful as high temperature (>42 C) results in wide gap between anthesis and silking ( ASI), Hence poor seed set. While very low temperature causes improper pollen shedding and seed setting. Therefore an optimum temperature of 21 C for germination 32 C for plant growth is suited.

**Seed Production Site**

Seed production should be taken in well drained ,weed and diseases free soil and preferably the fields where preceding crop was not maize to minimize roguing and maintain the genetic purity.

## Land Preparation

The land should be level, fertile, well drained and ph of 5.5 to 7.5 is congenial free from weeds and previous crop should not be maize on the same piece of land. Land should be brought to a good tilth with 2-3 ploughings and harrowing.

## Time of Sowing

Appropriate time of showing is very important for better crop establishment .For most part of India ,first week of July during kharif and first week of November during rabi are the optimum time of showing to avoid flowering from heavy rains during kharif and low temperature should not coincide with flowering .Rains during flowering wash the pollen in kharif and low temperature during winter causes mortally and killing another .

## Method of sowing and layout

It is desirable to plant the crop on ridges Sowing should be done on the southern side of the east-west ridges, which helps in good germination. Planting should be done at proper spacing. Optimum row and plant spacing should be kept at 60 and 20 cm, respectively. This spacing will ease the movement in the field for roguing and removal of tassels. Proper spacing also helps in improving the test weight. Identification labels/tags should be put on the male and female lines to distinguish between them.

The male line has to sown first for that the lines where male lines have to sown are to mark with a peg and later the female lines have to be sown in proper row proportions. All along the border 4 rows of male line has to be sown so that it will act as a natural barrier for pollen from other commercial / seed production Maize plots and also supply pollen Critical points to be taken note-

- Taking note of male and female seed bags
- Removing the tag keeping it safely for source verification along with the bill.
- Sowing the male lines first, at the marked lines.
- Then sowing the female lines
- Following proper cultural practice
- Removing of types at pre – flowering stage based on tassel colour, silk colour etc.
- Synchronization is not usually a problem in maize. However, if present spray 2% urea to late entry. Three methods of sowing are commonly followed under Indian conditions.



**Raised bed (ridge) planting**



**Furrow planting**



**Transplanting**

### Seed Rate

One should ensure that the seed viable and free from external infections. Information on seed germination should be obtained for deciding the quantity of seed to be used. Seed germination standard should be 99 to 100% and the quantity of seed required for dibbling method is about 15 kg/ha (female: 10 Kg/ha Male: 5kg/ha). The seed rate should be so adjusted as to obtain the desired plant population. The optimum plant population for achieving high yield is around 65 thousand plants/ha.

### Pest and diseases in maize (given in table-4)

**Table-4: Important Pest and diseases on Maize and their control measures**

| <b>Pest</b>     | <b>Control Measure by spraying</b>           |
|-----------------|--|
| Stem borer      | Quinolphos 25 EC -2 ml /ltr                  |
| Ear head bugs   | Carbary 14% -dusting                         |
| Army worm       | Nuvacron poison bait                         |
| Thrips & aphids | Dimethoate spray -1.7ml/ltr                  |
| <b>Disease</b>  | <b>Control Measure</b>                       |
| Rust            | Spray Zineb / mancozeb @ 2.5 gm/ltr of water |
| Leaf blight     | Seed treatment with metalaxzyl 3g/kg deed    |
| Downy mildew    | Ridomil MZspray                              |

### Field standards followed during seed production (Seed certification standards) of open- Pollinated Varieties, Synthetics and Composites

#### 1. Isolation

In seed production, three isolations, five isolations and seven isolations are required for single cross, three way cross and double cross hybrids respectively (Table). Hybrid crop raised from single cross at farmers' field is more uniform than double cross hybrid. Single cross seed production is a two stage process whereas double cross has

three stages. Due to all these factors, double cross seed production needs greater planning and coordination. Once the barrier of low seed yield of inbred lines are overcome, the single crosses are just natural among the conventional hybrids (Vasal et al. 1995).

### 1a. Isolation for OPV s, Synthetics and Composites.

The seed field shall be isolated from the contaminants shown in column 1 of the Table below by the distances specified in column 2 and 3 of the said in table 5:

**Table-5**

| Contaminants  | Minimum distance (meters) |           |
|---|---------------------------|-----------|
|   | Foundation                | Certified |
| Field of other varieties  | 400                       | 200       |
| Field of the same varieties not conforming to varietal purity requirements for certification and teosinte | 400                       | 200       |

**(Source: Indian seed certification standards, 2013)**

### 1b. Isolation for Hybrids is mentioned in table -6

**Table-6: Isolation blocks need if all three generations of seed multiplications are being taken at one place**

| Hybrid type                      |       | Number of isolations  |
|----------------------------------|-------|---|
| 1. Single cross (A × B)          | Three | First tow isolation for two inbreds, breeder seed and foundation seed production.<br>Third isolation for certified seed production (A×B).   |
| 2.Three-way cross (A × B) × C    | Five  | Three isolations for three inbreds (breeder and foundation seed production).<br>One isolation for F <sub>1</sub> seed production (A×B) as foundation seed. One isolation for production certified seed (A × B) × C                                  |
| 3.Double Top cross (A × B) × OPV | Five  | Three isolations for two inbreds and one OPV (breeder and foundation seed production).<br>One separate isolation for producing F <sub>1</sub> i.e., (A×B) crossed foundation seeds. One isolation for production of certified seed [(A × B) × OPV]. |
| 4.Double cross (A × B) (C × D)   | Seven | Four isolation for seed increase of the four inbred lines i.e. A,B,C and D (Breeder and foundation seed).<br>Two isolation for seed production of the tow parental single-cross hybrids (Foundation   |

|  |  |  |
|--|--|--|
|  |  | seed), i.e. (A×B) and (C×D).<br>One isolation for certified double cross hybrid<br>i.e. (A×B) × (C×D). |
|--|--|--|

A specific Hybrid of maize shall be isolated from the contaminants shown in column 1 of the table below by the distances specified in column 2 of the said Table -7 :

**Table-7:**

| <b>Contaminants</b>   | <b>Minimum distance (meters)</b> |
|---|----------------------------------|
| *Field of any maize with same kernel colour and texture   | 200                              |
| Field of any maize with different kernel colour and texture ,and teosinte   | 300                              |
| *field of the same hybrid (code designation)not confirming to varietal purity requirements for certification                              | 200                              |
| *field of the other hybrids having common male parent and conforming to varietal purity to varietal purity requirements for certification | 5                                |
| *field of the other hybrids having common male parent and not confirming to varietal purity requirements for certification                | 200                              |

**(Source: Indian seed certification standards, 2013)**

**Specific requirements of OPVs, synthetics and composites given in table -8**

**Table:8**

| <b>Factor</b>   | <b>Maximum permitted (%)</b> |                  |
|---|------------------------------|------------------|
|   | <b>Foundation</b>            | <b>Certified</b> |
| Off -type plants that have shed of shedding pollen at any one inspection during flowering when 5.0% or more of the plants in the seed field have receptive silks. | 1.0                          | 1.0              |

**(Source: Indian seed certification standards, 2013)**

**Specific requirements of Hybrids given in table-9**

**Table-9**

| <b>Factor</b>  | <b>Maximum permitted (%)</b> |
|--|------------------------------|
| Offtype plants that have shed or are shedding pollen in male parent at any one inspection during flowering when 5.0% of more of the plants in the seed field have receptive silks. | 0.50                         |
| Tassels of the plants that have shed or shedding pollen in seed parent at any one inspection during flowering when   | 1.00                         |



|   |      |
|---|------|
| 5.0% or more of the plants in the seed parent have receptive silks  |      |
| Total of pollen shedding tassels including tassels that have shed pollens for all three inspections conducted during flowering on different dates | 2.0  |
| Off-types plants in seed parent at final inspection   | 0.50 |

(Source: Indian seed certification standards, 2013)

## 2. Roguing

Roguing helps in maintaining the genetic purity of seeds. During the seed production of maize, strict roguing must be exercised. Fields are regularly inspected and off-types and doubtful plant are discarded before pollen is shed. Based on these observations the off type plants should be removed both in male and female lines before they shed the pollen. Normally, off-types and admixture plants are vigorous and easy to identify. Inbred lines, under of nodes, amount of chlorophyll, number of tassel branches, anther and silk colour, ear length, ear diameter, number of kernels per row and rows of ears kernel colour etc.



**Identifying dissimilar plant roguing**



**Pulling out dissimilar plant**

Generally roguing is done three times in maize. however, roguing is carried out depending upon the necessity. It is necessary to know the distinguishing features of the variety for effective roguing.

- First roguing should be done during vegetative stage, based on the height of the plant, colour of petiole and colour leaf.
- During flowering stage, second roguing is done based on colour of tassel and silk.
- Finally, before harvest, based on colour of seed and cob characteristics, roughing can be done.
- During drying of the cobs, roughing of cob based on seed colour and seed row will maintain the genetic purity.

**Attention:** During roguing at flowering stage, the off types should be removed away from the field immediately. Otherwise it will contaminate the silk and affect the genetic purity.

**Table-10 Characters to be observed at seed production plots**

| Characters | Parameters   |
|------------|--|
| Plant type | Height : tall/Dwarf<br>Stem: Pigmented / non pigmented   |
| Tassel     | Colour of glumes<br>Colour of anthers<br>Type : Compact or open<br>Silk Colour of silk : Green<br>pink /purple |
| Ear        | Type : Flint /semi flint /Dent<br>Colour : orange/yellow/yellow-orange<br>Cob: white pink                      |

### 3. Detasseling an Important Operation

Removing the tassel before it sheds the pollen from female lines in a seed production plot is known detasseling operation and it should be done before anthesis. It should be practiced row-wise. One person should follow to monitor the each row to check that no part of the tassel is left inside. The process of detasseling should continue for 8-10 days. While detasseling, leaf should not be removed which will otherwise reduce the photosynthesis. It has been observed that the removal of 1 to 3 leaves along with tassel reduces 5-15% yield. The removed tassel should not be thrown in the field but fed to the cattle as it is nutritive fodder.



**Ways of detasseling**

It is very important operation for hybrid seed production of maize. Procedure for this operation as follows:

- Tassel which are going to shed the pollen next day have to be identified.
- They have to be removed by pulling out the tassel out of the leaf whorl.
- The entire operation should be done during morning 7.30 to 10.00 a.m.
- The tassel removed should not be carried in the entire openly & care should be taken to for disposing the tassel.
- The entire operation should be done in 8-10 days and daily.

### 4. Male: female ratio

The male: female ratio depends on (a) pollen shedding potential and duration of male parent; (b) male: female synchrony :for better seed setting flowering of female

should be earlier than male or male pollen dehiscence should coincide with female silking and (c) season. In general the male: female ratio should be 1:2 or 1:3 or 1:4.

## 5. Field Inspection

Stages of crop inspection as follows:

**At the time of sowing purpose:** to monitor the land, isolation distance, planting ratio of male: female, proper sowing time, seed treatment etc.

**During pre flowering/vegetative stage purpose:** to verify the roguing and removal of off type plants (Photo 30 & 31)

**During flowering stage purpose :** to check disease and pest infestation

**During post-flowering and pre-harvest stage purpose:** to remove the late and diseased plants

**Harvesting time purpose:** to see the proper time of harvesting



Inspection at vegetative stages



Inspection at pre-flowering stages

**Seed standards followed during seed production (Seed certification standards) of open- Pollinated Varieties, Synthetics and Composites, and hybrids ( given in table-11)**

Seed ears inspected after harvest shall not contain in excess of 1.0% and 0.50% for Opvs, Synthetics, composites and Hybrids respectively of off -type ears including the ears with off -coloured kernels.

**Table-11**

| Factor                 | Standards for each class |           |
|------------------------|--------------------------|-----------|
|                        | Foundation               | Certified |
| Pure seed ( minimum)   | 98.0%                    | 98.0%     |
| Inert matter (maximum) | 2.0%                     | 2.0%      |

|   |       |       |
|---|-------|-------|
| Other Crop seeds(maximum)   | 5/kg  | 10/kg |
| Other distinguishable varieties based on kernel colour and texture ( maximum) | 10/kg | 20/Kg |
| Weed seeds (maximum)  | None  | None  |
| Germination ( minimum)  | 90%   | 90%   |
| Moisture  | 12.0% | 12.0% |
| For vapour –proof containers ( maximum)                                       | 8.0%  | 8.0%  |

## Hybrid seed production of OPVs, Synthetics, composites, and hybrids:

### Seed production of open pollinated varieties (OPVs):

Open pollinated variety of maize should be maintained in an isolation from other filed and roguing for plant characteristics should not be very strict as it may lead to random drift. Only off types, abnormal and disease and pest affected plants should be rogued. Population size of open pollinated variety should be maintained large with five thousand or more plants. Exact population size cannot be suggested accurately because it depends on the genetic makeup of a population. To make sure random mating in OPVs, 50 per cent of plants must be detasseled before pollination and it is practiced in every second row of field (is detasseled). Harvest and bulk the seed from detasseled plants only. Breeder, foundation and certified seed production should be in separate isolated large plots so that large scale random mating will takesplace and hence production of all genotypes in appropriate frequencies are possible.

## I. Nucleus seed production of synthetics, Composites varieties, and Hybrids:

### a. Synthetic varieties:

#### Synthesis of synthetic variety and nucleus seed production

Generally two methods can be followed to synthesis synthetic varieties.

- **Method I:** Equal number of seeds of each selected (based on general combining ability) line is mixed together and planted in an isolated plot under open pollination. Seed is harvested without selection for ear or plant types. The population raised from this seed is the synthetic-1 (syn-1)
- **Method II:** all possible crosses among the selected lines (based on general combining ability) are made in isolation. Equal numbers of seeds of each cross among the selected lines (if 8 lines (n) are there, a total of crosses will be  $n(n-1)/2$  i.e., twenty eight) are mixed together, and planted in an isolated plot. The population derived from this composite seed is known as the synthetic-1 (syn-1)  
The seed produced from syn-1 is used as nucleus seed which may be further used as a source seed for breeder seed production.

## **b. Composite varieties:**

### **Synthesis of composites and nucleus seed production:**

Choice of the material that should enter into the synthesis of a composite shall depend upon the objective for which it is built. In general, it would be desirable to include open pollinated varieties, synthetics, and advanced generation hybrid sets. Which have wide genetic diversity. Divergence is indicated by geographic origin or pedigree of material.

Selected lines, based on their performance, are grown in ear to row system. Chain crosses may be made among selected lines and resultant seeds mixed together on equal number and grown in isolation. About three to five cycles of intermating are required to homogenize. At harvest five to six best ears from each collection are saved for next cycle. Different methods of crossing, half sibbing, full sibbing, backcross and chain crosses among the population have been employed to develop composite varieties.

Indian maize programme had released a number of composites such as kisan, jawahar, vikram, sona, vijay, amber, pusa chandan and pusa kundan.

### **c. Hybrids: Nucleus seed production /Maintenance of inbred lines of**

To preserve the performance and uniformity of a particular hybrids, the same pure breeding inbred lines must always be used. Variation in certain traits within same inbreds, from separate sources of maintenance, has been observed by many workers. Changes in breeding behaviour of an inbred line may be due to : (i) delayed segregation (ii) Mutation (iii) out crossing and (iv) mechanical mixture.

The maintenance may be done by planting ear to row or by mixing seeds from ears of individual plant of inbred line for increase and may be maintained by selfing or full sib-pollination by hands. Self-pollination is the process of applying pollen of a plant to its own silks. Sib-pollination is the process of applying pollen of a plant to the silks of a sister plant (plant of the same line). Sibbing tends to prevent excessive loss of vigor and selfing increases homozygosity and uniformity. Very often, in many maintenance programmes parental lines are maintained by alternate selfing and sibbing from one generation to the next. This alternate system of selfing one year and sibbing the next year is more advantageous (Fleming and Kozelnicky 1965). In sibbing or selfing population size must be appropriate. Population size should be little more in inbred lines developed by a limiting number of inbreeding (early generation inbred lines). In another case, if inbred line was a resultant of large number of inbreeding cycle (advance generation inbred lines) relatively smaller population size may be sufficient. In other words, population size during maintenance mainly depends on the degree of variation present in identified inbred line. However, such a programme should not be limited to only few years. Contamination by foreign pollen must be avoided by maintaining desired isolation from all other maize plants.

## Hand Pollination

The maintenance or nucleus seed production of inbred lines generally involves hand pollinations. Cloudy, misty or rainy weather during flowering does not permit hand pollinations on a large scale. Therefore, either off-season sowing or sowing are adjusted to avoid undesirable weather conditions. As inbreds are poor in vigour, well-fertilized soil conditions and adequate spacing between plants are recommended.

Ears must be protected before silking with translucent paper bags called "silk bags" or isolator. The usual size of silk bags is 90×230 mm. The tassel bag may be used to cover the tassel of the same plant for selfing or other plant for sibbing. The tassel bag size should be 160 to 190 × 90 or 135 mm made out of brown paper. The date of bagging is marked in waterproof ink on the bag. This operation should be done before pollen shedding. For pollination, the paper bag isolator is removed from the ear and silk is shortened with pen-knife or scissors to about one cm. to facilitate even application of pollen the male plant is gently bent by holding it with the left hand in the flag leaf region and the tassel is tapped with the right hand to facilitate pollen shedding into the bag covering it. Crumple down the silk bag and slip the tassel bag over silk to be pollination and then pick up the bottom of the bag upwards and pollens are pumped around the silk and shake vigorously; this causes the pollen grains to fall upon the silks. Then the paper bag is folded at the bottom firmly around the stalk and fastened with a paper clip or stapler. This completes the pollination process. Care must be taken to avoid contamination with foreign pollen (Poehlman and Borthakur 1968).

## II. Breeder seed production:

### a. Synthetics

After a synthetic variety has been synthesized and nucleus seed is produced, the variety is maintained and multiplied by growing it in an isolated plot in subsequent years. Rouging may be done carefully and only undesirable (obvious off types) and diseased plants may be discarded, however, close selection on ear type is not advisable. Open pollination among all the plants is allowed sufficient random mating. The population derived from the seed Syn-1 is known as synthetic-2. Yield performance of synthetic-1 is better than synthetic-2 due to heterotic effects. Further, there will be no decline in yield of syn-2, syn-3, syn-4 and syn-5 due to zygotic equilibrium (Allard, 1960).

### b. Composites

Breeder seed of composite varieties may be increased in two stages as follow (Singh, 1987)

**Stage I:** five hundred or more half sibs should be carefully grown in isolation of the prescribed standard to produce the desired quantity of breeder seed for stage II. In half-

sib block all female rows are detasseled, the seed for male rows is formed through a balanced composite.

**Stage II:** seed should be grown in isolation. The seed source for stage II shall be drawn from stage I. ten to fifteen thousands plant population should be maintained. In fact, this size of population is necessary to avoid inbreeding depression and genetic drift. Row length should be of 5-10 meter length and rejection intensity in any individual cycle should not exceed 10-15 per cent.

### c. Hybrids

Breeder seed production means the increase of each inbred seed stock obtained from nucleus seed in an isolated field. Field should be free from volunteer plants. Roughing should be employed strictly before pollen shedding. Roughing should continue in pre-flowering, flowering and post-flowering stages also. Breeder seed production involving long-term inbred lines, which have limited variation, need special attention, particularly at the time of roughing. In short-term inbred, roughing should not be very strict. An experienced plant breeder/seed technologist, particularly who is well conversant with the variation in the inbred line should be employed for seed production. Frequent multiplication of the breeder seed to the extent possible, should be avoided in order to avert any likely drift in the basic population, particularly in cases where lines have limited degree of inbreeding. It is recommended that each seed multiplication should be of an order of 5-10 quintals, which may be used for two-three years. Different classes of seeds for different types of hybrids mentioned as follows

#### Different classes of seeds for different types of hybrids

| Type of hybrid          | Classes of seeds |  |
|-------------------------|------------------|--|
| Single cross            | Breeder seed     | Multiplication of two inbreds  |
|                         | Foundation seed  | Multiplication of two inbreds  |
|                         | Certified seed   | Production of hybrid seed (A X B)  |
| Three-way crosses       | Breeder seed     | Multiplication of three inbred lines   |
|                         | Foundation seed  | Multiplication of one inbred (C) and production of one F <sub>1</sub> i.e., (A x B). |
| Double top cross hybrid | Certified seed   | Production three-way hybrid (A x B) x C  |
|                         | Breeder seed     | Multiplication of two inbred lines and OPV.  |
| Double cross            | Foundation seed  | Multiplication of OPV and production of F <sub>1</sub> (A x B)                       |
|                         | Certified seed   | Production of three-way cross (A x B) x (OPV)  |
|                         | Breeder seed     | Multiplication of four inbreds   |
|                         | Foundation seed  | Production of two F <sub>1</sub> , i.e., (A x B) and (C x D)                         |
|                         | Certified seed   | Production of double cross i.e. (A x B)(C x D)                                       |

## **Monitoring of breeder seed plots**

The literary meaning of monitoring is "to observe critically". Generally four members team *i.e.* crop breeder, seed production breeder or maintainer, one officer from NSC and one officer from Seed Certification Agency where the seed is being produced visit the field at flowering time. The team evaluate the field for uniformity and varietal purity and submit their report on prescribed proforma.

Although there is no prescribed field standard for breeder seed production, however, monitoring team in their inspection report must mention that breeder seed crop under report is as pure as to guarantee that in subsequent generations *i.e.* certified foundation seed class shall conform to the prescribed standard of genetic purity.

## **III. Foundation seed production**

### **a. synthetics**

Before the seed is distributed to the farmers for commercial production, one or two cycles of seed multiplication should be done in an isolated field. By this time, population will reach to equilibrium. Selection or rouging should not be employed in a strict manner. If the production of seed is in sufficient quantity, then it may be distributed directly to the farmers.

### **b. composites**

The seed may be multiplied in prescribed isolated large size of plot following random mating. To avoid the undesirable out crossing or mechanical mixture, border rows may be raised from the same population. Selection pressure may be avoided, however, undesirable plant type and diseased plants must be discarded before pollen shedding. An intensive rouging may deviate the population from original genetic make up. Adequate plant populatin should be maintained.

### **c. Hybrids**

Foundation seed production is different for different types of hybrids as shown in Table 1. For foundation seed production breeder seed must be purchased from authentic source. As foundation seed is a certified class of seed, therefore, all precautions given by concerned certification agencies should be followed.

#### **1. Single-cross hybrids**

In case of single-cross hybrid (two parent hybrid) two inbred lines are raised in separate isolated field. Roguing and other cultural practices are the same as described in breeder seed production. Foundation seed of inbred lines will be used as source seed for the production of certified seed.



## 2. Three-way cross hybrids

For three-way cross hybrid, two isolated fields are required. One for producing the F<sub>1</sub> seed of single-cross (A x B) and one for single inbred lines. In crossing plot, the usual planting pattern is two rows of the female inbred (A) to one row of male inbred (B). Detasseling of female 'A' line is necessary, and seed is saved only from female rows. The seed multiplication of third inbred line is done as usual in an isolated field. As soon as pollination is completed in the crossing block (A x B), male line (B) should be harvested and removed from the field.

## 3. Double-cross hybrids

Foundation seed production of double cross hybrid means the production of two single cross involving four inbred lines (A, B, C and D). Two separate isolated plots are required to produce two single crosses i.e. A x B and C x D. Inbred A and C are detasseled when being crossed. However, breeder's advice must be obtained to identify female and male lines. Both crossed F<sub>1</sub> seeds should be clearly tagged with different colour labels indicating female and male F<sub>1</sub> seeds. This constitutes foundation seeds to produce certified (commercial) hybrid seeds. Selected fields for foundation seed (single cross) production need prescribed isolation distance (Table 2).

## 4. Double-top-cross hybrids

Two inbred lines i.e. A, B, and one open pollinated or composite population are involved in the production of foundation seed for double-top-cross hybrids. Two isolated plots are required, one for producing F<sub>1</sub> seeds of single-cross between A and B inbred lines and another for open-pollinated variety (Table 1). In crossing plot, one inbred is to be used as female or seed parent and another one as male. Usual planting ratio is two rows of female inbred A to one row of male inbred B. As usual detasseling is necessary in female line. The open pollinated variety may be increased in another plot in isolation.

## IV. certified seed production:

### a. synthetics

Foundation seed may be increased by one more cycle of production as certified commercial seeds. Each cycle of multiplication must be in a large plot for providing chances of random mating and producing plants of all frequencies. The deterioration in the variety may occur due to deviation from random mating and this could overcome by reconstituting the synthetic as necessary from the original sources.

### b. Composites.

Seeds may further be increased in an isolated plot as done in foundation seed production.

## **C. Hybrids:**

### **1. Single-cross hybrids**

In certified seed production plot, planting ratio of 1: 1 or 3: 1 is usually followed. Adequacy of pollen production is a primary consideration in determining the ratio of female to male parent. Being poor vigour in inbred lines low female: male ratio (2: 1 or sometimes 1 : 1 also) is used in single-cross between two inbreds to assure adequate pollination. Female line is detasseled when being crossed. Once the vigorous inbreds are available, single crosses are natural choice (Vasal 1995).

### **2. Three-way-cross hybrids**

Under certified seed production, foundation seed of one single-cross (A x B), female parent and one inbred to be taken as male parent are planted with ratio 3 : 1 or 4 : 1. Generally, three-way cross hybrid is produced where three inbred lines, which combine well, are available but fourth suitable inbred is lacking, otherwise, double cross is more economical. Single-crosses (female) are more vigorous, early and tall whereas inbred line (male) short and poor which is the major problem in poor seed set. Detasseling of female (A x B) is necessary.

### **3. Double-Top-cross hybrid**

One single-cross and one open-pollinated variety (OPV) or composite variety, are involved in this system. A hybrid seed from single cross is taken as female parent and OPV as male parent. Higher planting ratio of 8 : 2 or 10 : 2 may be used in double top cross-hybrid seed production as OPV or composites are more efficient pollen parents. Plants may vary in degree of variation within a OPV (heterogeneous population) and it is a task of crop breeders to encompass the acceptable range in the description of OPV. Therefore strict roguing is avoided. However, obvious off-types, diseased and poor plants should be rogued out.

### **4. Double cross hybrid**

Double-cross hybrids are the most widely used type of hybrids. The foundation seed of two single-crosses (A x B and C x D) may be procured from authentic source. One of these single-crosses is to be used as the female parent and other single cross as male Parent. In fact, in each hybrid, male and female parent is pre-decided by the breeder. The single-cross A x B is detasseled when being crossed to C x D. The male parent is also a single cross hybrid and capable to shed more pollen for pollination. Therefore, the planting ratio between female and male parent may be increased to 8:2 or 10:2. The seed production of double-cross hybrid is quite cost effective. The double-cross hybrid crops are relatively more variable for plant characters than single-cross hybrid crop, which may be an advantage when crop is grown under adverse conditions. If necessary, a higher plant density of the male rows may be used and sowing the male rows could be undertaken on two dates in two very closely spaced adjacent rows to

ensure better pollen availability for longer duration, (Singh 1987). Tunwar and Singh (1988) have prescribed the isolation distance requirements for certified seed production (Table 2).

### **Harvesting and threshing (Under the supervision of Certification agency staff)**

Male parent should be harvested first than the female and should be kept separately. Optimum moisture content in grain at harvesting should be around 20%. The harvested cobs should be spread evenly instead of making heap.

- The male lines should be cut immediately after the pollination is over
- Female lines should be harvested when completely dry and the ear heads should be sorted out
- The ear heads should be dried in threshing yard (up to 12%)
- May be dipped in melathion solution, dried and threshed
- Grading the seeds , discarding the small and malformed seeds

### **Shelling**

Shelling of female parent should be done earlier than male to avoid mechanical mixture. Shelling can be done manually or by power operated maize Sheller.

### **Seed processing, Storage and marketing**

All under size, broken, damaged etc seeds should be removed for maintaining the quality of seed. Seed drying should be done till the moisture content of the seed is reduced to 8 % and it should be kept in aerated jute bags. Seed should be stored at cool and dry place preferably in cold storage. Poor storage conditions will lead to loss of vigour and poor germination. Marketing should be done with specifications and standards.

### **Seed Yield**

The hybrid seed yield depends upon the type of hybrid seed produced e.g. Single cross hybrid mean the seed yield is low ( 8-10 qt/ac) as the parent is an inbred line and Hybrid seed yield will be more if it is three way cross hybrid of double cross hybrid (15 Q /Ac) as the female parent is already a F1 hybrid .

### **Conclusion**

To check the depleting ground water in country and to maintain the soil fertility by suggesting alternative remunerative crops to replace at least one third area from rice to other less water requiring but profitable crops like maize. Maize can solely contribute towards shifting area under rice cultivation and an action plan is going on with policy makers of our country. Production of hybrid maize seed is a unique and dynamic industry worldwide. Many steps are involved in the production, processing, and marketing chain. This work is often done under contract with private farmers, thus the selection, training, and collaboration with the best farmers is essential. Once the seed crop has been harvested it must be transported to the processing facility where it is de-

husked, dried, sorted, cleaned, sized, treated, and packaged. The emphasize that Single Cross Hybrid breeding program and to meet the nutritional security of maize eating population, the QPM would remain the focussed area of research and seed production programs would receive greater attention in this direction which will provide more employment, good yield and higher food grain production. So th joint venture of India and ASEAN countries will mutually benefitted through sharing of expertise in the filed oaf agriculture with special emphasize on maize seed production programme to feed their maize eating population.

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## Hybrid Rice Seed Production -An overview

Hybrid rice is the proven technology for increasing rice production and productivity and with good management, yield advantage of 1.0 – 1.5 t/ha can be obtained by cultivation of hybrids as compared to the high yielding varieties under the same set of growing conditions. In the Asia Pacific region, where more than 80% of rice is produced and consumed, hybrid rice cultivation can play an important role in increasing the productivity in the region. The potential and usefulness of this technology in enhancing rice productivity is clearly evident from the fact that the few countries in the region (China, India, Vietnam) are leading in hybrid rice cultivation.

In India, very good progress has been made in the hybrid rice research and development and as a result of intensive research efforts over the last two decades, 66 hybrids with high yield potential and better grain quality have been released for commercial cultivation (Table 1). About 30-35 of them are in the active seed production chain in the country and by encouraging the cultivation of these hybrids in the country, rice production and productivity can be improved. During the year 2012, hybrid rice was planted in an area of 2.0 m.ha. and an additional rice production of 2.5 to 3.0 m.t. was added to our food basket through this technology. More than 80 % of the total hybrid rice area is in Uttar Pradesh, Jharkhand, Bihar, Chhattisgarh, Punjab and Haryana. Our country has the distinction of developing and releasing first Basmati quality rice hybrid Pusa RH-10 (developed at IARI, New Delhi) and it has become very popular in north India.

The seed production technology has been perfected over the years and many progressive farmers recorded more than three tonnes of hybrid seed yield per hectare. It is estimated that every year more than 25000-30000 tonnes of hybrid rice seed is being produced in India and more than 95% of hybrid rice seed production activities are in the hands of private seed companies. Private seed sector along with public sector research network is playing an important role in the popularization of hybrid rice technology. Through their effective marketing and distribution network, private seed companies are facilitating the availability of hybrid rice seed to the farming community.

Seed production is one of the crucial components to translate the benefits of crop improvement efforts to the farming community. Production of pure seed is as important as developing the improved variety/ hybrid. Pure seed can be obtained only from a pure crop. Pure crop is characterized by high germination, uniformity in growth, crop stand, flowering and maturity. These factors depend upon the genetic purity. According to an estimate, the yield of hybrid crop decrease about 100 kg/ha with every 1% decrease in the purity of hybrid seed. High genetic purity of the parental lines is a pre-requisite to ensure the purity of hybrids which in turn helps to realize their full genetic potential.

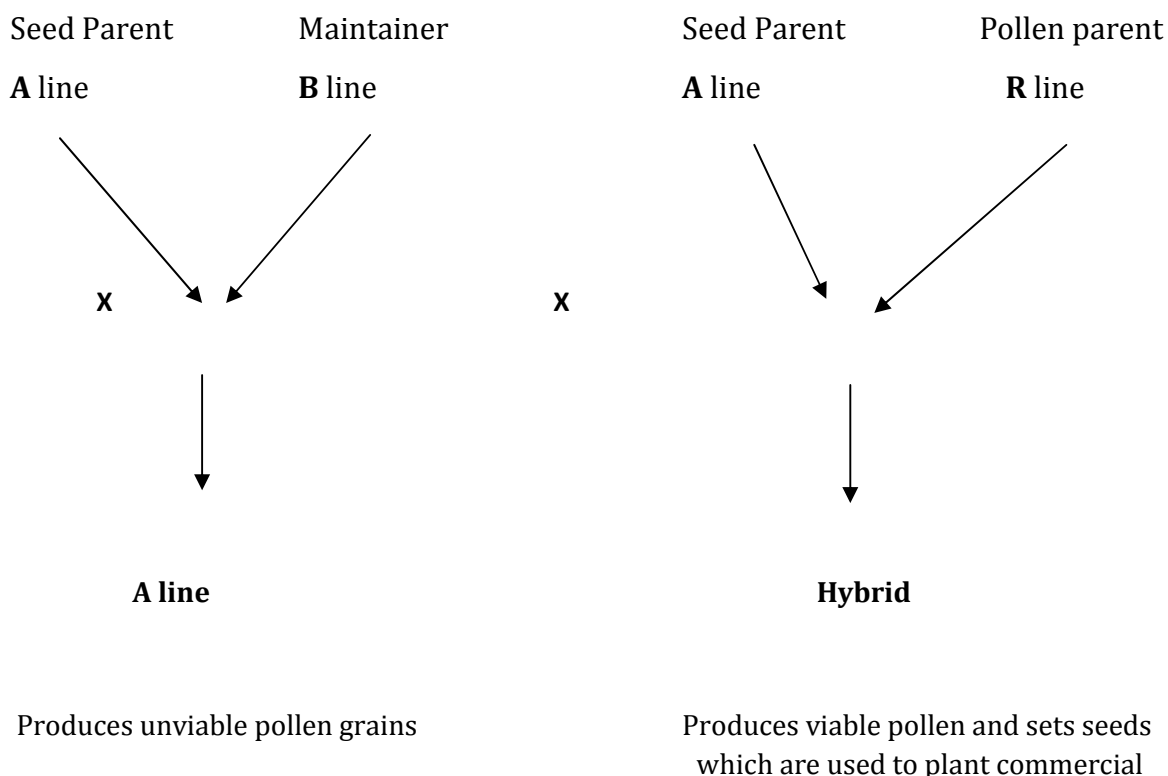
Over a period of time, parental lines degenerate due to mechanical mixtures arising out from inept handling, biological mixtures due to unwanted cross pollination,

natural mutations and genetic segregation. All these factors individually or jointly degenerate the parental lines which may affect the seed quality. Hence the parental lines are required to be regularly purified by adopting the standard chain of Nucleus seed, Breeder seed and Foundation seed in a systematic manner directly under the supervision of concerned breeder.

The existing rice hybrids used in commercial production in India are developed by using cytoplasmic genetic male sterility and fertility restoration system (CMS system). This system involves three lines viz., cytoplasmic genetic male sterile line (CMS or 'A' line), maintainer ('B' line) and restorer ('R' line) lines for developing rice hybrids. Hybrid Seed Production using the CMS system involves the following two steps.

- Production of 'A' line (A x B)
- Production of Hybrid Seed (A x R)

The 'B' and 'R' lines are multiplied in the same way as inbred varieties.



Success of Hybrid Rice Technology depends on efficient and economic seed production on large scale. It determines whether the heterosis of hybrid rice can be fully exploited or not. It is reported that the yield of F<sub>1</sub> hybrids will decrease by 0.8% when the seed purity decreases by 1%, so it is very important to establish a sustainable system of seed production to ensure the purity of hybrid seeds in hybrid rice development.

Thus the procedure of hybrid rice seed production, in which two different lines including male sterile lines (seed parent) and restorer lines (pollen parent) are planted alternatively in a certain row ratio in the same field and the outcrossed seeds are harvested from the male sterile plants, differs from that of inbred varieties, in which only one line is grown and the selfed seeds are harvested. Therefore, in the whole process of hybrid seed production, it requires a set of complicated techniques centering on raising the out crossing rate to obtain a high seed yield.

Rice is self-pollinated crop, where the extent of natural out crossing is only 0.3 to 3.0%. Therefore hybrid rice seed production requires specialized techniques, which need to be thoroughly understood before embarking upon this venture. The success of hybrid seed production depends on various factors such as choice of field, isolation, seeding time, planting pattern and weather conditions during the period of flowering, roguing synchronization in flowering of parental lines, supplementary pollination techniques, proper harvesting, processing, packing and effective seed distribution etc.

### **1. Choice of location:**

Choosing a desirable location for hybrid seed production is very important. In the well isolated area, the paddy field with fertile soil, a desired irrigation and drainage system, sufficient sunshine, and no serious disease and insect problems are essentially needed.

### **2. Isolation:**

Rice pollen grains are very small and light, and can travel very far with the wind. In order to ensure the purity of hybrid seed and avoid pollination by unwanted rice varieties, the hybrid seed production plots should be strictly isolated by the following methods.

#### **Space isolation**

A space isolation of 50 – 100 m would be satisfactory for hybrid seed production, which implies that within this range no other rice varieties should be grown except the pollen parent.

#### **Time isolation**

Wherever, it is difficult to have space isolation, a time isolation of over 21 days would also be effective. It means that the heading stage of the parental lines in hybrid seed production plot should be 21 days earlier or later than that of other varieties grown within the vicinity.

#### **Barrier isolation**

In some places, the natural topographic features such as mountains, rivers, forests can serve as the most effective barrier. A crop barrier with maize, sugarcane, sesbania covering a distance of 30 m would also serve the purpose of isolation. Artificial

barrier with polythene sheets of about 2 m height can also be used for small scale seed production. However, the most ideal locations are the areas covered with hillocks and mountains, which act as natural barriers.

### 3. Favorable climatic conditions:

Climatic conditions have profound influence on the seed yields. Detailed information on the weather data of a given locality is necessary for fixing the seeding dates. Seeding of the parental lines should be planned in such a way that the flowering coincides with the most favorable climatic conditions, which are as follows:

- Daily mean temperature of 24 – 30° c
- Relative humidity ranging from 70 – 80 %
- The differences between day and night temperatures should not be more than 8–10°c, preferably 5 – 7°c
- Sufficient sun shine with moderate wind velocity.
- There should not be rains continuously for three days during the period of flowering.
- Seed yields will be adversely affected if the temperature is below 20°c and above 35°c.
- The Seed Production areas near forest, rivulets and valleys are better for getting higher seed production.

In India, most of the hybrid rice seed is produced during dry season in Karimnagar and Warangal districts of Andhra Pradesh. The weather conditions prevailing in the Warangal district in dry season are as follows (Table 2):

**Table 2: Weekly weather data of Warangal district – averaged for 15 years**

| Month | Week | Rh (%) | Mean Temp °C | Difference in day and night temperatures°C | Rainfall(mm) |
|-------|------|--------|--------------|--|--------------|
| 2     | 1    | 84.50  | 25.43        | 9.98                                       | 2.20         |
| 2     | 2    | 81.86  | 26.07        | 9.84                                       | 0.56         |
| 2     | 3    | 79.27  | 26.77        | 10.42                                      | 0.74         |
| 2     | 4    | 75.10  | 29.06        | 9.72                                       | 0.00         |
| 3     | 1    | 75.73  | 30.59        | 9.77                                       | 0.00         |
| 3     | 2    | 76.86  | 29.25        | 10.23                                      | 0.10         |
| 3     | 3    | 75.83  | 28.65        | 10.90                                      | 0.19         |
| 3     | 4    | 78.02  | 30.46        | 10.77                                      | 0.36         |

### 4. Seeding of parental lines in the seedbed

- Puddle the seedbed field properly. Puddle the field twice at an interval of 6-7 days to destroy weeds, weed seeds and germinated rice seeds.



- Prepare raised seedbeds (5-10 cm height) of 1m width of any convenient length.
- Provide drainage channels in between seedbeds to drain excess water.
- Apply recommended fertilizer to the nursery beds
- Sow pregerminated seed uniformly on the seedbed (1-2 kg seed/20m<sup>2</sup>)
- Use 15 kg of 'A' line seed and 5 kg of 'R' line seed to produce sufficient seedlings to grow one hectare.
- Manage the seedbed properly for getting healthy and vigorous seedlings for transplanting.

## 5. Transplanting

Commence transplanting seedlings of A and R lines as and when they attain the age of 21-25 days, which ensures timely heading, and flowering of parental lines. Transplanting of older seedlings delays flowering and transplanting of younger seedlings advances flowering. If the transplanting of seedlings of 'A' line is delayed, then delay transplanting the 'R' line seedlings by the same number of days to synchronize flowering. Transplant one or two seedlings per hill of the 'A' line and two seedlings per hill of 'R' lines.

**5.1 Transplanting in a specific Row Ratio & Row direction:** In hybrid rice seed production the seed parent and pollen parent are planted in a certain row ratio at certain spacing. The row ratio and spacing of pollen parent and seed parent have a distinct effect on the hybrid seed yields.

The row ratio or row proportion refers to the number of rows of the male parent (R line) to that of the female parent (A line) in a seed production plot. Suppose if we plant 2 rows of 'R' line followed by 8 rows of 'A', the row ratio can be taken as 2:8. In hybrid rice seed production plot the recommended male (R) to female (A) row ratio is 2:8. However, the row ratio may vary from region to region, depending on weather, management and parental lines. R and A lines can be planted in several row ratios of 2:8; 2:12; 3:10 etc.

**5.2 Factors Influencing Row Ratio:** The ratio of pollen parent (R line) to seed parent (A line) is determined by the characteristics of the parental lines.

- Plant height of pollinator
- Growth and vigour of the pollinator
- Size of the panicles and amount of residual pollen
- Duration and angle of floret opening in CMS lines
- Stigma exertion of CMS lines

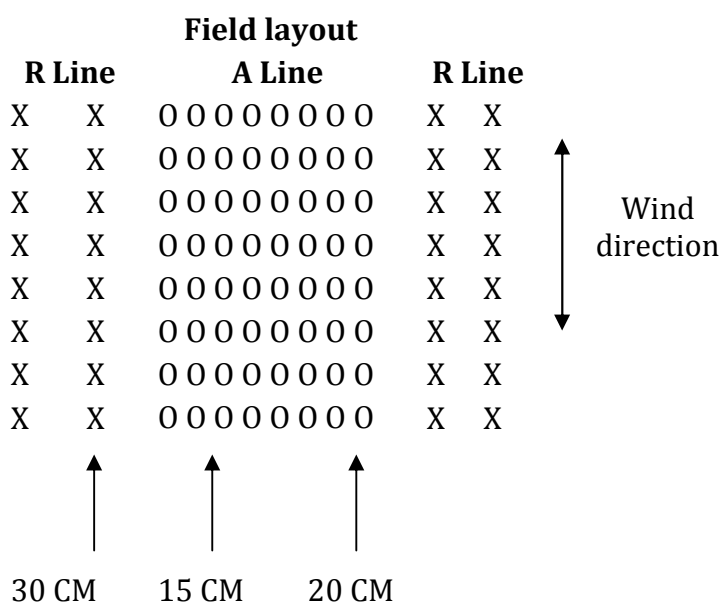
To facilitate out crossing, the rows of male and female in the seed production plot should be perpendicular to the prevailing wind direction expected at flowering time of the parents.

### 5.3 Transplanting of the R line

- Transplant the seedlings of R line in paired rows
- Leave a space of 145 cms inside block between paired rows of `R` line seedlings for transplanting 8 row blocks of `A` line seedlings.
- Transplant 2-3 seedlings per hill with a row-to-row distance of 30 cms and plant-to-plant spacing of 15 cms.

### 5.4 Transplanting of CMS line (A line)

- Transplant `A` line seedlings in blocks of 8 rows in between the paired rows of `R` lines
- Transplant with 1-2 seedlings per hill at a spacing of 15 x 15 cms
- Leave a 20 cms wide alleyway between A line rows and nearest R line row.



#### Spacings:

|                                  |               |
|----------------------------------|---------------|
| Between `R` line rows            | : 30 cms      |
| Between `A` line rows            | : 15 cms      |
| Between `R` & `A` line blocks    | : 20 – 30 cms |
| Between hills ( `A` & `R` lines) | : 15 cms      |
| Row Ratio                        | : 2R: 8A      |

### 5.5 Transplanting Sequence

The transplanting sequence of seed parent and pollen parent in the hybrid rice seed production plot depends on the growth duration of seed parent (A line) and pollen parent (R line)

**5.5.1 Seed parent (A line) has 10 day longer growth duration than pollen parent (R line):** Transplant 25day old seedlings of the `A` line, 10 days earlier than the second `R` line seedlings. The seedlings of the R line are transplanted when the seedlings from

the second R line seeding are 25 days old. At this time the age of seedlings from the first R line seeding will be 21 days old and the age of seedlings from third R line seeding will be 29 days old.

Seeding Sequence and seedlings age for transplanting

| S. No. | Seed/pollen parent | Seeding sequence | Seedling age for transplanting (days) |
|--------|--------------------|------------------|---------------------------------------|
| 1      | A line             | 0 day            | 25                                    |
| 2      | First R line       | 6th day          | 21                                    |
| 3      | Second R line      | 10th day         | 25                                    |
| 4      | Third R line       | 14th day         | 29                                    |

**5.5.2 Seed parent (A line) has 10 day shorter growth duration than pollen parent (R line):** The seedlings of the R line are transplanted when the seedlings from the second R line seeding are 25 days old. At this time the age of seedlings from the first R line seeding will be 21 days old and the age of the seedlings from the third R line seeding will be 29 days old. Later transplant 25 days old seedlings of the A line 10 days later than the second R line seedlings.

Seeding Sequence and seedlings age for transplanting

| S. No. | Seed/pollen parent | Seeding sequence | Seedling age for transplanting (days) |
|--------|--------------------|------------------|---------------------------------------|
| 1      | First R line       | 0 day            | 21                                    |
| 2      | Second R line      | 4th day          | 25                                    |
| 3      | Third R line       | 8th day          | 29                                    |
| 4      | A line             | 14th day         | 25                                    |

**5.5.3 Seed parent (A line) has same growth duration as pollen parent (R line):** The planting of both R and A lines can be done simultaneously. First complete the A line plantings with 25 day old seedlings followed by R line plantings with the seedlings ages of 21day old first R line, 25 days old second R line and 29days old third R line.

Seeding Sequence and seedlings age for transplanting

| S. No. | Seed/pollen parent       | Seeding sequence | Seedling age for transplanting (days) |
|--------|--------------------------|------------------|---------------------------------------|
| 1      | First R line             | 0 day            | 21                                    |
| 2      | Second R line and A line | 4th day          | 25                                    |
| 3      | Third R line             | 8th day          | 29                                    |

## 6. Roguing

The purity of hybrid rice seeds used in commercial production must be more than 98%. To meet this requirement, the purity of the restorer and CMS lines must be more than 99%. Therefore, in addition to ensuring strict isolation, it is necessary to remove all rogues from the seed production plots. Roguing is the removal of undesirable rice plants from the hybrid seed production plots. Undesirable rice plants are those plants either in A or R line rows that differ from plants that are true to type. Roguing helps to prevent the off-types from cross pollinating the true to type A line plants and thus enhancing the purity of hybrid seed.

The undesirable plants come from many sources. They may be voluntary plants from the previous crop. Contamination due to improper isolation also result in the occurrence of off-types. Admixing during the process of harvesting, threshing, packing and handling are also other sources from which the off-types occurred. Therefore, due care is to be taken to remove the off-types during the cropping season.

Roguing can be done at any time during the crop stage. Off-type rogues can be removed whenever they are identified – earlier the better. The most important stages for roguing are at maximum tillering, flowering and just before harvesting.

**6.1 Roguing at maximum tillering:** We can identify the off-types by their morphological differences from the true to type plants. Therefore, it is essential to know the characteristic features of parental lines, which help in easy identification of rogues and efficient roguing. As a basic step, any plant found outside the rows has to be removed as they may be volunteer plants. Remove all those plants which are either too tall or too short than the seed or pollen parent. We can also identify the off-type plants by difference in their leaf blade size, shape and leaf sheath colour.

**6.2 Roguing at flowering:** Roguing at flowering is extremely important as it is the stage when we can identify many off-types which look similar to the parental lines during the early stages of growth. All the off-type plants that flower very early or very late are to be removed. The plants which differ from parental line plants in respect of leaf size, shape, angle, panicle shape, size and pigmentation are to be carefully removed. Remove all the plants from A line that have plumpy yellow anthers. Plants in the A line should not have fertile pollen. The off-types in A lines can also be distinguished from their fully exerted panicles. Care should be taken to remove the plants which are highly infested from pests and diseases.

**6.3 Roguing just before harvest:** This is the last opportunity to keep away the off-types in order to maintain high purity. Before harvesting, the plants in A line rows are to be thoroughly checked and those plants which show normal seed set are to be removed. It is necessary to remove all the off-types that have different grain characters as

compared to that of A line plants. The grain size, shape, colour and pigmentation of A line plants have to be critically examined for effective roguing.

## **7. Promotion of exertion of panicle:**

Most of the male sterile lines based on WA cytoplasm have imperfect exertion of panicle, with the result as much as 15% spikelets remain enclosed in the flag leaf and are not exposed for out crossing. Through following methods, the exertion of the panicles can be promoted to a great extent.

**7.1 Application of gibberellic acid (GA<sub>3</sub>):** It is an efficient and effective growth hormone, which stimulates the cell elongation, thus can be used to enhance panicle exertion in CMS line. Besides, GA<sub>3</sub> has the following favorable effects:

- i. Increases the duration of floret opening
- ii. Increases stigma exertion and receptivity
- iii. Promotes plant height
- iv. Influences flowering and hence flowering in parental lines can be adjusted
- v. Widens the flag leaf angle
- vi. Promotes exertion and growth rate of secondary and tertiary tillers.

In hybrid seed production plots of rice, 5-10% panicle emergence stage is most appropriate for first spraying (40%) and the remaining 60% of GA<sub>3</sub> should be sprayed on the following day. The ideal time for spraying is from 8 to 10 AM and from 4 to 6 PM. Spraying should be avoided during cloudy weather and when the wind velocity is high. A dose of 45-60 g/ha of GA<sub>3</sub> is optimum. The hormone does not dissolve in water and it should be dissolved in 70% alcohol (1 g of GA<sub>3</sub> in 25-40 ml of alcohol).

**7.2 Flag leaf clipping:** Normally the flag leaves are erect and longer than the panicles and they come in the way of easy pollen dispersal thus effecting the out crossing rate. The clipping of flag leaf helps in free movement and wide dispersal of pollen grains to give higher seed production. The flag leaves should be clipped when the main culms are in booting stage. Only half or two-third portion of flag leaf should be removed. However, flag leaf cutting is not advisable in the plots infested with diseases as this operation may spread the disease further.

## **8. Supplementary pollination:**

Rice is basically a self-pollinated crop and hence there is a need to go for supplementary pollination in order to enhance the extent of out crossing. Supplementary pollination is a technique of shaking the pollen parent so that the pollen is shed and effectively dispersed over the A line plants. Supplementary pollination can be done either by rope pulling or by shaking the pollen parent with the help of two bamboo sticks. Timing and frequency of supplementary pollination is very important. The first supplementary pollination should be done at peak anthesis time i.e. when 30-

40 % of the spikelets are opened. This process is repeated 3 – 4 times during the day at an interval of 30 minutes. Supplementary pollination has to be done for 7-10 days during the flowering period.

## **9. Harvesting, threshing and processing**

From the point of view of maintaining high purity, extreme care is needed while harvesting, threshing and processing of the hybrid rice plots.

**9.1 Harvesting:** Harvest all R lines rows first. Remove the R line harvest and keep it in a safe place separately. Carefully remove the left over R line panicles in the field.

**9.2 Threshing:** During threshing, the 'A' line parent and 'R' line parent harvests must be kept separate from each other. The A and R lines should be threshed separately. Before starting threshing, all the threshing equipment, threshing floor and tarpaulin to be thoroughly cleaned.

Use new gunny bags for storing the seeds. Prepare two labels for each bag – one to place inside the bag and one to attach to the bag outside. Each label should contain the following information.

1. Name and Address
2. Name of the parent
3. Name of the location
4. Season and year
5. Date of harvest

### **9.3 Seed drying:**

- Seed drying helps seeds maintain their ability to germinate and their vigour for a longer period.
- Drying controls mold growth and the activity of the other organisms, that reduce the quality of stored grain
- Drying reduces seed discoloration
- Seeds can be safely stored when they have been dried to a moisture content of less than 13 %.

**9.3.1 Seed drying methods:** Seeds can be dried by two methods viz., sun-drying and forced air-drying.

**Sun drying:** The seeds can be dried by placing them on jute bags or on a tarpaulin. Do not dry the seeds directly on the concrete threshing floor. While drying, stir the seeds occasionally to ensure uniform drying.

**Forced air-drying:** Seeds can be dried in a batch – type dryer by forced air heated to 40-45°C. The seed layer in a batch type drier should not be more than 45 cm deep. Dry the seeds slowly and do not dry abruptly to 13% moisture content.

**9.4 Seed Processing:** Seed Processing has to be done to remove impurities like trash, leaves, broken seeds sand etc., weed seeds and to remove immature, shriveled, unfilled and empty spikelets.

Seed processing usually done by public and private seed agencies by using Air screen machines. Air screen machines in addition to cleaning the seeds, grading also will be done by separating the seeds of uniform size from over size and under size seeds.

### **Seed certification Standards – Hybrid Rice**

General seed certification standards are basic and, together with the following specific standards constitute the standards for certification of hybrid rice seed.

#### **A. Eligibility Requirements:**

- i. An inbred line to be eligible for certification shall be from a source such that its identity may be assured and approved by the Certification Agency.
- ii. Hybrid seed to be eligible for certification shall be the progeny of two approved inbred lines, one of which shall be male sterile

**B. Classes and Sources of Seed:** An inbred line shall be relatively a true breeding strain resulting from self-pollination with selection.

- i. The foundation class seed shall consist of an approved male sterile line to be used as a female parent and an approved inbred line to be used as a male parent for the purpose of producing hybrid seed.
- ii. A male sterile line shall be a strain A carrying cytoplasmic-genetic male sterility, which sheds no viable pollen and is maintained by the normal sister strain B which is used as a pollinator.
- iii. The certified class seed shall be the hybrid seed to be planted for any use except seed production.

#### **C. Land Requirements, Field Inspection and Field as well as Seed standards:**

i) Land Requirements: Land to be used for production of hybrid rice seeds must be free of volunteer plants or self-sown seeds.

ii) Field Inspection: For the purpose of certification, a minimum of four inspections are required at different stages as indicated below.

- The first inspection shall be made before flowering in order to determine isolation, presence of volunteer plants, outcrosses, planting ratio, errors in planting and other relevant factors;

- The second and third inspection shall be made during flowering to check isolation, offtypes, pollen shedders in female parent and other relevant factors;

i) Field Standards:

### A. General Requirements:

#### 1. Isolation:

- Hybrid rice seed fields shall be isolated from the contaminants shown in column 1 of the table below by the distances specified in columns 2 and 3 of the said table.

| Contaminants  | Minimum distance (meters) |           |
|---|---------------------------|-----------|
|   | Foundation                | Certified |
| Fields of other varieties including commercial hybrid of the same variety                                 | 200                       | 100       |
| Fields of same hybrid (code designation) not conforming to varietal purity requirements for certification | 200                       | 100       |

### B. Specific requirements:

| Factors                                 | Maximum permitted (%)* |           |
|---|------------------------|-----------|
|   | Foundation             | Certified |
| Offtypes in seed parent                 | 0.050                  | 0.20      |
| Offtypes in pollinator                  | 0.050                  | 0.20      |
| Pollen shedding panicles in seed parent | 0.050                  | 0.10      |
| ** Objectionable weed plants            | 0.10                   | 0.020     |

\* : Standards shall be met at any inspection conducted at and after flowering.

\*\* : Objectionable weed shall be wild rice (*oryza sativa* L. var. *fatua*.)

### IV) Seed Standards:

The following certification standards would essentially be required to be met:

| Factors   | Standards for each class |           |
|---|--------------------------|-----------|
|   | Foundation               | Certified |
| Pure seed (minimum)                             | 98.0%                    | 98.0%     |
| Inert matter (maximum)                          | 2.0%                     | 2.0%      |
| Huskless seed (maximum)                         | 2.0%                     | 2.0%      |
| Other crop seed (maximum)                       | 10/kg                    | 20/kg     |
| Other distinguishable varieties seeds (maximum) | 10/kg                    | 20/kg     |
| Total weed seeds (maximum)                      | 10/kg                    | 20/kg     |



|  |                |                |
|--|----------------|----------------|
| Objectionable weed seeds (maximum)   | 2/kg           | 5/kg           |
| Seeds infected by paddy bunt ( <i>Neovossia horrida</i> Tak.) Padw. And Khan (maximum) | 0.10% (by No.) | 0.50% (by No.) |
| Germination (minimum)  | 80%            | 80%            |
| Moisture (maximum)   | 13.0%          | 13.0%          |
| For vapour-proof containers (maximum)  | 8.0%           | 8.0%           |

## Conclusions

A good beginning has been made by ushering in to an era of hybrid rice in the country. Development of heterotic hybrids by the researchers, large scale production of hybrid seeds by various seed agencies and transfer of this technology to the end users by the extension agencies must go hand in hand to have the real impact of this technology in the Indian agriculture. Though the hybrid rice technology has been introduced to Indian agriculture, the successful large scale adoption of this innovative technology, in future, primarily depends upon the economic attractiveness of this technology. Rice hybrids with still higher magnitude of heterosis coupled with better grain, cooking and eating quality and possessing resistance to major pests and diseases needs to be developed.

Seed production technology has to be further refined to obtain average seed yields of 2.5 to 3.0 t/ha on a large scale, so that the cost of hybrid rice seed can be reduced to Rs. 100/- kg. Top priority has to be given to maintain the purity of parental lines and to produce high quality hybrid seed. Involvement of seed agencies in the public sector, NGO's and farmers cooperatives along with the private seed sector will be crucial to meet the increased demand for hybrid seed in the years to come.

**Table 1: List of hybrids released in India (1994-2013)**

| S. No. | Hybrid                | 50% flowering duration (DFF) | Year of Release | Notification No. | Date of notification | Developed by                           | Released for the states of   |
|--------|-----------------------|------------------------------|-----------------|------------------|----------------------|--|--|
| 1      | APHR-1                | 100                          | 1994            | 662(E)           | 17.9.1997            | APRRI, Maruteru (ANGRAU), Hyderabad    | Andhra Pradesh   |
| 2      | APHR-2                | 90                           | 1994            | 662(E)           | 17.9.1997            | APRRI, Maruteru (ANGRAU), Hyderabad    | Andhra Pradesh   |
| 3      | MGR-1 (CORH-1)        | 85                           | 1994            | 360(E)           | 1.5.1997             | TNAU, Coimbatore                       | Tamil Nadu   |
| 4      | KRH-1                 | 95                           | 1994            | 1(E)             | 1.1.1996             | ZARS, VC Farm, Mandya (UAS, Bengaluru) | Karnataka  |
| 5      | CNRH-3                | 95                           | 1995            | -                |                      | RRS, Chinsurah, West Bengal            | West Bengal  |
| 6      | DRRH-1                | 100                          | 1996            | 401(E)           | 15.5.1998            | DRR, Hyderabad                         | Andhra Pradesh   |
| 7      | KRH-2                 | 100                          | 1996            | 401(E)           | 15.5.1998            | ZARS, VC Farm, Mandya (UAS, Bengaluru) | Pondicherry, Bihar, Karnataka, TN, Tripura, Maharashtra, Haryana, Orissa, Uttaranchal, Rajasthan and West Bengal |
| 8      | Pant SankarDhan -1    | 90                           | 1997            | 425(E)           | 8.6.1999             | GBPUA&T, Pantnagar                     | Uttar Pradesh  |
| 9      | PHB 71                | 100                          | 1997            | 647(E)           | 9.9.1997             | Pioneer overseas corp. Hyderabad       | Haryana, UP, TN, A.P. Karnataka  |
| 10     | CORH-2                | 95                           | 1999            | 425(E)           | 8.6.1999             | TNAU, Coimbatore                       | Tamil Nadu   |
| 11     | ADTRH-1               | 85                           | 1999            | 425(E)           | 8.6.1999             | TNRRI, Aduthurai (TNAU)                | Tamil Nadu   |
| 12     | Sahyadri              | 100                          | 1998            | 821(E)           | 13.9.2000            | RARS, Karjat (BSKKV)                   | Maharashtra  |
| 13     | NarendraSankar Dhan-2 | 98                           | 1998            | 425(E)           | 8.6.1999             | NDUA&T, Faizabad                       | Uttar Pradesh  |
| 14     | PA 6201               | 95                           | 2000            | 92(E)            | 19.7.2000            | Bayer Bio-Science, Hyderabad           | A.P., Karnataka, TN, Bihar, Orissa, Tripura, U.P. W.B. and M.P.  |

| S. No. | Hybrid                    | 50% flowering duration (DFP) | Year of Release | Notification No. | Date of notification | Developed by                            | Released for the states of   |
|--------|---------------------------|------------------------------|-----------------|------------------|----------------------|---|--|
| 15     | PA 6444                   | 105                          | 2001            | 1134(E)          | 5.11.2001            | Bayer Bio-Science, Hyderabad            | UP, Tripura, Orissa, AP, Karnataka, Maharashtra and Uttarakand           |
| 16     | Pusa RH-10                | 95                           | 2001            | 1134(E)          | 15.11.2001           | IARI , New Delhi                        | Haryana, Delhi, UP, Uttarakand.  |
| 17     | Ganga                     | 100                          | 2001            | 599 (E)          | 25.4.2006            | Paras extra growth seeds Ltd. Hyderabad | Uttarakand, Punjab, Nagaland, Haryana, U.P., Orissa, Bihar               |
| 18     | RH-204                    | 95                           | 2002            | 283(E)           | 12.3.2003            | Parry Monsanto seeds Ltd. Bengaluru     | AP, Karnataka, TN, West Haryana, Uttarakand and Rajasthan                |
| 19     | Suruchi                   | 103                          | 2004            | 122(E)           | 2.2.2005             | Mahyco Ltd, Aurangabad                  | Haryana, Andhra Pradesh, Gujarat, Chattisgarh, Karnataka and Mahrashtra. |
| 20     | Pant Sankar Dhan-3        | 92                           | 2004            | 599 (E)          | 25.4.2006            | GBPUA&T, Pantnagar                      | Uttarakand   |
| 21     | NarendraUsarSankar Dhan-3 | 105                          | 2005            |                  |                      | NDUA&T, Faizabad                        | Saline and alkaline areas of U.P.  |
| 22     | DRRH-2                    | 86                           | 2005            | 1566 (E)         | 5.11.2005            | DRR, Hyderabad                          | Haryana, Uttarnachal, West Bengal and Tamil Nadu.                        |
| 23     | Rajlaxmi                  | 98/128(Boro)                 | 2005            | 1572 (E)         | 20.9.2006            | CRRRI , Cuttack                         | Orissa and Boro areas of Assam   |
| 24     | Ajay                      | 98                           | 2005            | 1572 (E)         | 20.9.2006            | CRRRI , Cuttack                         | Orissa   |
| 25     | Sahyadri-2                | 85                           | 2005            | 122 (E)          | 6.2.2007             | RARS, Karjat (BSKKV)                    | Maharashtra  |
| 26     | Sahyadri-3                | 95                           | 2005            | 122 (E)          | 6.2.2007             | RARS, Karjat (BSKKV)                    | Maharashtra  |
| 27     | HKRH-1                    | 104                          | 2006            | 122 (E)          | 6.2.2007             | RARS, Kaul (CCSHAU)                     | Haryana  |
| 28     | JKRH-401                  | 110                          | 2006            | 122 (E)          | 6.2.2007             | JK Agri Genetics Ltd, Hyderabad         | West Bengal, Bihar, Orissa   |
| 29     | CORH-3                    | 85                           | 2006            | 1178 (E)         | 20.7.2007            | TNAU, Coimbatore                        | Tamil Nadu   |
| 30     | Indira Sona               | 98                           | 2006            | 1178 (E)         | 20.7.2007            | IGKV, Raipur                            | Chhattisgarh   |

| S. No. | Hybrid     | 50% flowering duration (DFD) | Year of Release | Notification No. | Date of notification | Developed by                          | Released for the states of                       |
|--------|------------|------------------------------|-----------------|------------------|----------------------|---------------------------------------|--|
| 31     | JRH-4      | 87                           | 2007            | 1178 (E)         | 20.7.2007            | JNKVV, Jabalpur                       | Madhya Pradesh                                   |
| 32     | JRH-5      | 87                           | 2007            | 1178 (E)         | 20.7.2007            | JNKVV, Jabalpur                       | Madhya Pradesh                                   |
| 33     | PA 6129    | 85                           | 2007            | 1703 (E)         | 5.10.2007            | Bayer Bio-Science, Hyderabad          | Punjab, T.N., Pondichery.                        |
| 34     | GK 5003    | 88                           | 2008            | 454 (E)          | 11.2.2009            | Ganga Kaveri seeds Pvt Ltd, Hyderabad | Andhra Pradesh and Karnataka                     |
| 35     | Sahyadri 4 | 88                           | 2008            | 454 (E)          | 11.2.2009            | RARS, Karjat (BSKKV)                  | Maharashtra, UP, Punjab, Haryana and West Bengal |
| 36     | JRH-8      | 90                           | 2008            | 449(E)           | 11.2.2009            | JNKVV, Jabalpur                       | Madhya Pradesh                                   |

## Seed Quality Assurance During Storage–Seed Health Management

Heavy losses have been recorded in the course of seed storage throughout the world. Importance of the additional seed that can be made available for production and consumption by pest free/ pest controlled storage is intensely felt during unfavourable agricultural years. Surplus grains produced in the country only aggravate storage problems. Increasingly, attention is being focused on minimizing storage losses depending on available knowledge. In the past fifty years, a number of research reviews and books have been published in the field of biology, bionomics and control in stored seeds through various gadgets.

More than 65 per cent of the total grain produced is retained and stored by the farmers for food, feed and seed purposes till the next harvest. It is estimated that about 5-8 per cent of grains are retained for seed, about 20 per cent is hand pounded and the rest is milled.

To the present level of production, an additional 150 million tonnes food grain production has to be achieved to feed almost 1.5 billion people by 2040. Thus, our efforts to augment production of food grain, have to be supported with strategies to lower the storage losses.

Many think, the damage to the seeds occur during storage only; hence management of insect pests in storage only is targeted. In spite of proper management, the damage in storage continues to be enormous. This is so, because some of the major storage pests have been observed to infest/multiply under field conditions, which are carried over to storage along with the harvested grains. The losses during threshing (1.68%), transportation (0.15%), processing (0.92%) and storage (6.58%) amount to almost 8-9 per cent.

Seed security is key to the attainment of household food security among resource poor farmers in developing countries. Seed is a valuable commodity. Like most biological materials, seed is also vulnerable to many factors that can cause deterioration during storage. Temperature and relative humidity of the storage environment, damage from insects or rodents can all contribute to loss of seed quality.

Majority of storage pests recorded in India belong to orders Coleoptera and Lepidoptera. Based on the nature of damage and their feeding habit, they are grouped as internal feeders and external feeders.

## A. Internal feeders

**1. Weevils :**                      **Family:** Curculionidae                      **Order:** Coleoptera

| Scientific names                 | Common names      |
|----------------------------------|-------------------|
| 1. <i>Sitophilus oryzae</i> (L.) | Rice black Weevil |
| 2. <i>S. Zeamais</i> (Motsch)    | Maize weevil      |
| 3. <i>S. granaries</i> (L.)      | Granary weevil    |

### Rice Weevil, *Sitophilus oryzae* (L.)

#### Identification

- Size of an adult is around 3 mm with long snout or beak
- The body colour appears to be brown or black
- On close observation, four orange / red spots are arranged in cross on the wing covers
- It can able to fly.



### Maize Weevil, *S. Zeamais* (Motsch)

#### Identification

- Close relative of rice weevil with 2.5 to 4 mm size
- Small brown weevil has four reddish brown spots on the wing covers (elytra).
- It has a long, thin snout and elbowed antennae
- It appears similar to the rice weevil, but has more clearly marked spots on the wing covers and is somewhat larger.
- It can able to fly.



### Granary weevil, *S. granaries* (L.)

#### Identification

- Adult weevils are about 3 to 5 mm size with elongate snout.
- The adults are radish brown in colour and lack distinguishing marks.
- It cannot able to fly



### Nature of damage

Both grubs and adults cause the damage, seeds hollowed out, kernels are reduced to mere powder; *Sitophilus oryzae* and *S. Zeamais* are known to attack grains in fields too. Adults cut circular holes. Heating takes place during heavy infestation, which is known as dry heating.



### Commodities damaged

Wheat, rice, maize, sorghum and paddy (rarely)

### 2. Lesser Grain Borer

| Scientific Name                    | Common Name  | Family:      | Order:     |
|------------------------------------|--|--------------|------------|
| <i>Rhyzopertha dominica</i> (Fab.) | Lesser Grain Borer,<br>Hood Grain Borer,<br>Paddy Borer Beetle | Bostrichidae | Coleoptera |

### Identification

- Adults are 3-4 mm in size
- Large pronotum appearing to have only two body sections
- It has got 3 distinct antennal clubs with last 3 segments are larger than the other segments.
- The shape of the posterior area of the elytra is rounded.



### Nature of damage

Irregular holes (c.f.: Weevils) in bagged storage, irregular messy waste flour spots indicate infestation of this pest. Heating is very common. Localized infestation is almost a rule. Both adults and larvae cause damage and are voracious feeders. Seed kernels are reduced to mere shells. The damaged kernels remain engulfed in a film of waste flour.



### Commodities damaged

Paddy, rice, wheat, maize, millets, coriander, oats, chilli and turmeric. It attacks paddy more easily than *S. oryzae*.

**3. Pulse beetles**      **Family:** Chrysomelidae, Bruchinae      **Order:** Coleoptera

| Scientific names                     | Common names  |
|--------------------------------------|---------------|
| <i>Callosobruchus maculatus</i> (F.) | Pulse beetles |
| <i>C. chinensis</i> L.               |               |
| <i>C. theobromae</i>                 |               |
| <i>C. analis</i>                     |               |

### Identification

#### *Callosobruchus maculatus* (F.)

- Small, short, active with long conspicuous serrate antennae, 3 to 4 mm in size.
- Colour is reddish brown, slightly elongated beetle
- Wing covers are marked with black and gray with two black spots near the middle.



#### Pulse Beetle, *Callosobruchus chinensis* L.

- It is 3 to 4 mm in size long oval shaped chocolate coloured body it is pointed towards front.
- The size of the adult beetle depends on the size of the infested pulse.
- There are dark patches on elytra and thorax.
- Elytra do not cover the abdomen completely.



#### Nature of damage

Larvae eat up the grain, kernel and make a cavity. Adults are short lived, harmless and do not feed on stored produce at all. The infestation starts from the field itself because the beetle can actively fly.



### Commodities attacked

Practically whole pulses such red gram, greengram, blackgram, bean, cowpea, soybean.



#### 4. Grain moth

| Scientific name                          | Common Name          | Family      | Order       |
|--|----------------------|-------------|-------------|
| <i>Sitotroga cerealella</i><br>(Olivier) | Angoumois grain moth | Gelechiidae | Lepidoptera |

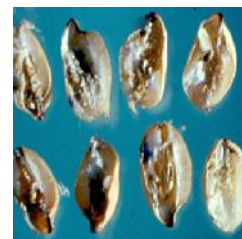
#### Identification:

- Size 8 to 10 mm. Moth yellowish brown with wings completely folded over back in sloping manner. Wing expanse 10-14 mm.\
- Hind wings with sharp pointing apical end and bearing heavy fringe of bristles.
- Leaves small dirty specks on window pans and walls.



#### Nature of damage

It is a primary pest. Only larvae damage grains, adults being harmless. Grains are hollowed out. It attacks in field and stores too. In stored bulk grains, infestation confined to upper 30cms depth only. Hole is circular with characteristic 'flap' or 'trap door'.



#### Commodities attacked

Paddy, maize, sorghum, barley and wheat (rarely). It is not capable of attacking milled rice or other cereal products.

#### 5. Tamarind beetle

| Scientific name                       | Common Name                           | Family                         | Order      |
|---------------------------------------|---------------------------------------|--------------------------------|------------|
| <i>Caryedon serratus</i><br>(Olivier) | Tamarind beetle,<br>Groundnut bruchid | Chrysomelidae,<br>Pachymerinae | Coleoptera |

#### Identification:

- Adult beetle is robust brunched. Reddish brown cuticle densely clothed with grey brown setae but with dark markings on the elytra
- The pygidium in the female fully visible from above.
- Each hind femur bears a conspicuous ventral comb of one large spine and 8 to 12 smaller ones.
- The full grown larvae are migratory in nature and reddish brown and fleshy



### Nature of damage:

Larvae bore into the groundnut is revealed by larval emergence holes and the presence of cocoons outside the pods. Feeding damage to the seeds can be seen when infested pods split open.



### Commodities attacked

Tamarind pods, Groundnut pods, wild tree legumes like *Cassia*, *Acacia*, *Bauhinia*, etc.

## B. External Feeders

### 1. Khapra beetle

| Scientific name                    | Common Name   | Family      | Order      |
|------------------------------------|---------------|-------------|------------|
| <i>Trogoderma granarium</i> Everts | Khapra beetle | Dermestidae | Coleoptera |

### Identification

- Adult size 1.5 to 3mm, convex, oval in shape with practically no distinct division of head, thorax and abdomen. Abdomen size is comparatively larger.
- Sexual dimorphism is well developed, males being smaller in size than females.
- Larvae are straw coloured with dark brown hairy bands on each segment and typical posterior tuft forming a trail of long hairs, size 0.5 to 5mm



### Nature of damage

Being a primary pest, it damages the grain starting with germ portion, surface scratching and devouring the grain. Actually it reduces grain into frass. Excessive moulting creates public loss and market appeal due to insanitation caused by the cast skins, frass, and hair. Crowding of larvae leads to unhygienic conditions in warehouses. Damage is confined to peripheral layers of bags to a depth of 30 to 50 cm in bulk storage.



### Commodities attacked

Wheat, maize, sorghum, rice, pulse, oilseeds and their cakes.

## 2. Rust red Flour beetle

| Scientific name   | Common Name                                       | Family        | Order      |
|---|---|---------------|------------|
| <i>Tribolium castaneum</i><br>(Herbst.)<br><i>T. confusum</i> Duval | Rust red flour beetle<br>Confused flour<br>beetle | Tenebrionidae | Coleoptera |

### Identification:

- *Tribolium castaneum* is reddish brown and antennae end with 3 segmented club.
- *T. castaneum* is same in colour, antennae end in a gradual club, the club consisting of four segments.



### Nature of damage:

Both adults and larvae feed on milled products. Flour beetles are secondary pests of all grains and primary pests of flour and other milled products. In grains, embryo or germ portion is preferred for feeding.



### Commodities attacked:

Groundnut, oats, lima bean, rice barley, walnuts, peas etc.

## 3. Saw toothed grain beetle

| Scientific name                       | Common Name              | Family     | Order      |
|---------------------------------------|--------------------------|------------|------------|
| <i>Oryzaephilus surinamensis</i> (L.) | Saw toothed grain beetle | Silvanidae | Coleoptera |

### Identification:

1. Narrow, flattened, 2.5-3.0 mm long
2. Thorax having teeth like serrations on each side
3. Antennae clubbed
4. Elytra cover abdomen completely
5. Adults are winged but they rarely fly



### Nature of damage

Adults and larvae cause roughening of grain surface and off odour in grain. Grains with higher percentage of broken, dockage and foreign matter sustain heavy infestation which leads to heating of grain.



### Commodities damaged

Rice, wheat, maize, cereal, products, and dry fruits.

### 4. Warehouse moth

| Scientific name          | Common Name        | Family     | Order       |
|--------------------------|--------------------|------------|-------------|
| <i>Ephesita cautella</i> | Fig or almond moth | phycitidae | Lepidoptera |

#### Identification:

- Size 13 mm, wings expanse 2.0- 2.5 mm
- Wings are dirty white in colour with distinct black bands about 4 mm from the head
- It rests with sloped wings over the body almost like the slanting roof of warehouses
- Being nocturnal, rests in dark places during day time. It sometimes flies during day time also; usually it is active at dust when temperature and R.H. fluctuations occur.



### Nature of damage

Only larval stage is harmful. It mainly feeds on germ portion leaving the rest of the kernel undamaged. In bulk infestation, its damage is limited to peripheral top layers only. Web formation covers the bags, floor space and mill machinery thereby leading to clogging in mills.

### Commodities attacked

Wheat, rice, maize, sorghum, groundnut, spices

### 5. Indian meal moth

| Scientific name                          | Common Name    | Family     | Order      |
|--|----------------|------------|------------|
| <i>Plodia interpunctella</i><br>(Hubner) | Meal worm moth | Phycitidae | Coleoptera |

### Identification:

- Fore wing basal half silver white or greyish, outer 2/3 portion is reddish, copper bronze lustre with irregular bands.
- Hind wings long, silvery, darker with reddish scales
- Thorax is slightly darker with reddish scale
- Hind tibia robust
- When the insect is at rest, antennae 2/3 of the body size cross and rest on the wings



### Nature of damage

Primary pest, cause serious damage to ear and grains of maize, contaminates the grains with excrement, cast skins, webbing, dead individuals and cocoons; prefers to eat germ portion and hence grains lose viability.



### Commodities Attacked

Maize, cereals, dry fruits, groundnuts and cereal products

### 6. Rice moth

| Scientific name                          | Common Name | Family      | Order       |
|--|-------------|-------------|-------------|
| <i>Corcyra cephalonica</i><br>(Stainten) | Rice moth   | Galleriidae | Lepidoptera |

### Identification:

- Spot free informally pale buff brown colour
- Wing expanse 25 mm
- Fore wings with dark views
- Cocoons dense white and tough
- Larvae with conical prolegs on abdominal segments



### **Nature of damage**

Larva is only responsible for damage. It pollutes food grains with frass, moults and dense webbing, in case of whole grains, kernels are bound into lumps up to 2 kg .



### **Commodities Attacked**

Rice, sorghum, other millets whole cereals, cereal products, dals, processed products of cereals, pulses, oil seeds, nuts, dry fruits and milled spices.

### **Sources of infestation of stored products by insects**

The belief is that, the insect outbreak in stored products is spontaneous cannot be true as stored grain insects, originate chiefly from indigenous insect populations. The main sources of infestation are

- i. Field infestation
- ii. Infestation by migration
- iii. Infestation through conveyance
- iv. Infestation through storage building or structure, bird's nests and ant's, rodent burrows, etc.

### **Impact on Seed Quality:**

Many of the storage insect pests were known to feed on the embryo of the seed which results in germination failure ultimately, the seeds are unfit for sowing. ISTA organisation has fixed minimum standard for insect infestation. They have fixed 0.5% damage for cereals and 1.0% damage for pulses. If it crosses more then the prescribed damage the lot will be rejected for seed purpose. However, experiments carried at our centre revealed that even after 4 percent damage, the germination was above 96% in both cereals as well as pulses, which clearly indicates their is need for modification of standards fixed by ISTA.

### **Management of insect pests of stored seeds**

#### **Control of insect pests**

Amongst the present methods of insect control, following are the important methods which can help in safe storage of seeds particularly at farmer's level.

1. Preventive measures
2. Curative measures

## Preventive measures

"Prevention is better than cure" hence the following preventive measures are recommended

### Hygiene or sanitation

1. Threshing floor /yard should be clean, free from insect infestation and away from the vicinity of villages and granaries.
2. Clean the harvesting and the threshing machines before their use.
3. Trucks, trolleys or bullock carts which are used for transportation of seeds should be made free from insect infestation.
4. Clean the storage godowns / structures before storage of newly harvested crop.
5. All dirt, rubbish, sweepings and webbings should be removed from the stores and disposed / destroyed.
6. All the cracks, crevices, holes existing in the floors and ceilings should be plastered with mud or cement permanently.
7. All the rat burrows should be closed with a mixture of broken glass pieces and mud and then plastered with mud/ cement.
8. White wash the store rooms before storage of seeds.
9. Seeds should be kept in stores which are rat & moisture proof.
10. Proper stacking of bags also helps in seed protection.
11. Proper handling of seeds and avoiding hooks on storage bags help minimize exposure to insects.
12. Bags should be stacked on wooden dunnage 0.5 meter away from the wall.
13. Bags should be stacked in rows having space of nearly 2 to 3 meters in between height of a row should not be more than 15 bags leaving about 1/5<sup>th</sup> space of total storage from the roof.
14. Bulk storage structures of seeds should also be kept away from the ventilators or doors.
15. Seed crop harvested at a moisture content ranging from 20 – 28 per cent should be dry to safe moisture content
16. Sun drying and use of mechanical dryers can be opted to bring down moisture.
17. Improper drying of seeds during post harvest operations enhances the insect infestation.
18. Staggered sun drying with short exposure to sun spread over large number of days (9 to 11 am for 8 days) reduces insect infestation.
19. Use of improved storage structures with gunny bags or jute bags with close weaves can reduce insect infestation.
20. Impregnation of gunny bags with insecticides can prevent entry of insects and polythene lined gunny bags were suggested by polyester-polythene 400 gauge lined canvas was found to be resistant to all types of insect attack.

### **Disinfestations of stores / receptacles**

Before the use, the receptacles / store rooms should be disinfested with approved residual insecticides preferably by spraying a Malathion 50% EC, with a dilution of 1: 100 and applied at the rate of 3 lit /100 m<sup>2</sup> or dichlorovos @ 1ml/ litre of water and seal the store house for a week without allowing the air to enter.

### **Fumigation**

- Decide the need for shed fumigation (entire store house or godown) or cover fumigation (only selected blocks of bags).
- Check the store house / godown and the black polythene sheets or rubberized aluminium covers for holes and get them ready for fumigation.
- Choose the fumigant and work out the requirement based on the following guidelines.
- Fumigation with Aluminium phosphide @ 3 tablets of 3 g each per tonne of seeds and for shed fumigation @ 21 tablets of 3 g each for 28 cubic meters for the period of 5 days.
- Insert the required number of aluminium phosphide tablets in between the bags in different layers. Cover the bags immediately with fumigation cover. Plaster the edges of cover all round with wet red earth or clay plaster or use sand snakes to make leak proof. Keep the bags for a period of 5-7 days under fumigation. Remove the mud plaster after specified fumigation period and lift cover in the corner to allow the residual gas to escape. Allow aeration and lift cover after a few hours. Follow similar steps in case of shed fumigation also.

### **Curative measures**

The infestation of stored seed insect pests can be controlled by following methods.

- I. Non- chemical control measures
- II. Chemical control measures.

#### **1. Non- chemical control measure**

The measures where chemicals are not used for control of insect pests of stored seeds are

#### **Ecological control measures**

The infestation of stored seeds from insect pests largely depends on the proper management of three factors *viz.*,

- i. Temperature



- ii. Moisture content of seeds
- iii. Availability of oxygen

All these factors are required for normal rapid development and multiplication of insects in the godowns and storage practices. These storage practices can be modified to create ecological conditions unfavourable for attack by insects.

### Temperature

Temperature ranging from 20<sup>o</sup> C to 40<sup>o</sup> C, accelerates the development of insects but above 42<sup>o</sup>C and below 14<sup>o</sup>C retards reproduction and development, while prolonged temperature above 45<sup>o</sup> C below 10<sup>o</sup>C may kill the insects. Heating of seeds at 50<sup>o</sup>C will be lethal to insects but it is not advisable because the seeds are affected and lose their viability.

### Moisture content of seed

Moisture is the critical factor in safe storage of seeds. The seeds stored at around 10 per cent moisture content escape from the attack of insects (except khapra beetle.)

Advisable moisture content of seeds for safe storage

|                 |          |
|-----------------|----------|
| Cereals         | : 10-12% |
| Pulses          | : 9-10 % |
| Oilseeds        | : 7-8 %  |
| Vegetable seeds | : >7%    |

It is desirable to know the moisture content of seed lots just after harvest or before storage as an aid to seed trade. The role of moisture in life of a seed is given below

| Seed moisture content (%) | Effect on Seed  |
|---------------------------|---|
| 18-40                     | Seed physiologically mature. Seed susceptible to field deterioration, moths and insects very active.            |
| 13-18                     | Moths and insects can be damaging seed  |
| 10-13                     | Seed store reasonably well for 6-18 months in open storage. Insects can still be a problem in susceptible seed. |
| 8-10                      | Very little insect activity, seed very susceptible to mechanical damage.  |

|     |   |
|-----|---|
| 4-8 | Safe moisture content for sealed storage    |
| 0-4 | Extreme desiccation can be damaging to seed |

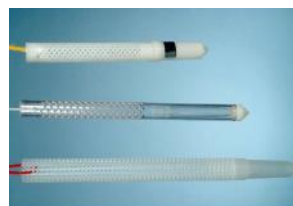
### Availability of oxygen

In storage, oxygen is consumed by seeds and insects during respiration and carbon dioxide is produced. Insects respire at the rate of 20,000 to 1,30,000 times than that of the same weight of the seeds. Thus, O<sub>2</sub> level will reduce below 1 % and CO<sub>2</sub> level will automatically increase which will be lethal to all the stages of insects.

### Traps to monitor the incidence of storage pests:

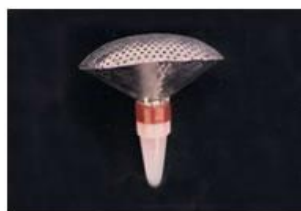
#### Probe Traps

The use of traps is a relatively new method of detecting insects in bulk stored seeds or in bag storage. Probe traps are used by keeping them under seed surface. The traps are simple to use, escape proof to many species and provide a mechanism for continuous monitoring of stored product insects. The trap can be inserted into the stored seeds and left for two weeks for best results. Insects like *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, *Oryzaephilus surinamensis* and *Sitotroga cerealella* can effectively be trapped and monitored by probe traps.



#### Pulse beetle trap

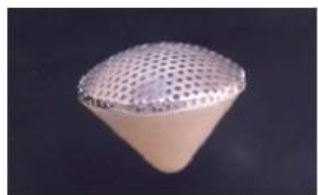
This is also a probe trap type model specially designed for pulse beetle. The traps can be kept with the cover lid alone protruding out of the seed. As generally, the emerging beetles come to the top for free movement and mating. Restricting the placement of traps on the side and centre of the storage bin or container will be sufficient to collect them.



## Pitfall Traps

Pitfall traps have been used widely to capture soil inhabiting insects. These traps can logically be use in bulk stored seeds for capturing insects active on the seed surface.

Crawling insect pests enter the trap by crawling or falling through the holes in the top. Generally, special coatings with sticky material like tangle foot on the inside of the cone on which the insects cannot walk and holds the insect inside the trap.



## Light Traps

Electrophysiological and behavioural studies have shown that majority of stored products insects responds to light of wave length between 300-700 nm. Stored product insects give their largest response to light of 350 nm (UV) and 500- 550 nm (Green light.) Green light is mostly preferred by stored product Lepidopterans. Lesser grain borer *Rhyzopertha dominica*, Red flour beetle, *Tribolium castaneum*, Cigarette beetle, *Lasioderma*, *Corcyra* etc., are attracted to UV light in large numbers. Lepidopterans like *Sitotroga cerealella*, *Corcyra cephalonica* etc., prefer green light source. UV light traps can be used for the beetles attacking paddy which are attracted in large numbers to UV light. Significant among them are *Rhyzopoertha* and *Tribolium castaneum*.



## Sticky traps

Sticky boards, screens, paper strips, hollow tubes, wing traps have been used to trap stored product moths. Sticky traps have been widely used in food processing plants. Various models of sticky traps have been developed for use in monitoring aerial populations of stored product insects. However, sticky traps are generally used with pheromones. Several sticky trap models for monitoring stored product insects are available.



## Pheromone traps

Pheromone traps of various models have been designed for use in pest management of various stored pests. Pheromone lures are available for many stored product insects namely, *E. cautella*, *S. cerealella*, *C. cephalonica*, *R. dominica*, *T. castaneum*, *O. surinamensis* and *T. granarium*. In general, in Lepidopteran moths, females produce chemical substance which attracts males. But fortunately, in major families of stored product beetles, the adult males release chemicals commonly termed as aggregation pheromones to which both females and males get attracted. Examples for this include *T. castaneum*, *T. confusum*, *R. dominica* and *O. surinamensis*. The value of these pheromones in stored product management has clearly been demonstrated by many workers.



## II. Chemical control measures

### a. Prophylactic treatments of seeds

- The produce is meant for seed purpose, mix 1.0 kg of activated kaolin or 1.0 kg of lindane 1.3 D or 1.0 kg of malathion 5 D for every 100 kg of seed and store / pack in gunny or polythene lined bags.
- To protect the pulse seeds, mix activated kaolin at the above dosage or any of the edible oils at 1.0 kg for every 100 kg seeds or mix 1.0 kg of neem seed kernel powder for every 100 kg of cereal or pulse seeds.

### b. Curative seed treatments

#### 1. Inorganic chemicals

- Treat the seeds of cereals or pulses used for seed with deltamethrin 40 mg/kg of seed by diluting insecticides in 5 ml of water before treating and shade dry for few minutes, and then store the seeds by packing them in polylined gunny bags.

**National seed Project, University of Agricultural Sciences, Bangalore recommended the following seed treatment insecticides to manage the storage insect pests of seed**

- Treat the seeds of cereals or pulses used for seed with bifenthrin 20 mg/kg of seed by diluting insecticides in 5 ml of water before treating and shade dry for few minutes, and then store the seeds by packing them in polylined gunny bags.
- Treat the seeds of pulses used for seed purpose with Emamectin benzoate 5 SG @ 40 mg or Spinosad 45 SC @ 0.4 ml diluted in 5 ml of water per kg of seed to control the pulse beetle in storage upto 12 months. Treat the seeds of maize used for seed purpose with spinosad 45 SC @ 0.4 ml mixed in 5 ml of water per kilogram of seed to control the storage insect pests of maize upto nine months.
- Treating the groundnut pods with deltamethrin 2.8 EC @ 1ppm (0.04 ml) or thiodicarb 75 WP @ 2 ppm (2.70 mg) or spinosad 45 SC @ 2 ppm (0.04 ml) by diluting in 15 ml of water, shade drying and storing the pods in gunny bags for management of groundnut pod borer under ambient conditions up to 9 months without affecting the seed quality parameters.
- The groundnut pods packed in High Density Poly Ethylene (HDPE) bags treated with deltamethrin 2.8 EC @ 100 ppm (3.5ml /liter) or spinosad 45 SC @ 100 ppm (0.2ml/liter) for management of pod borer *Caryodon serratus (Olivier)* upto nine months of storage under ambient conditions without affecting the seed quality parameters.

**2. Organic seed treatments**

- Neem based insecticides containing 3000 ppm of azadirachtin content for cereals and 10,000 ppm of azadirachtin content for the pulses at the rate of 5 ml/kg seeds were also recommended for the management of storage pests.
- Maize seeds treated with vasambu (*Achorus calamus*) dry rhizome powder @ 10 g / kg seed and packed in 400 gauge polythene bags is recommended to store the seeds safely for more than one season.

## **New Dimensions In Seed Certification: OECD Scheme**

Improved varieties and good quality seeds are inevitable to confront the challenges of ever increasing population and food insecurity. Improved seed is a carrier of technological innovations and serves as an engine for agricultural advancement when available in the required quantities and of the right quality. Seed being a commodity of trade, seed production supply activities and seed quality constitutes a more serious source of concern than seed quantity. As the first input in the cropping process, high quality seed brings high genetic yield potential resulting in higher productivity and crop production.

Seed certification is a legally sanctioned system for the quality control of seed during seed multiplication and production. The main objective of the Seed certification is to ensure the acceptable standards of seed viability, vigour, purity and seed health. A well organized seed certification should help in accomplishing systematic increase of superior varieties, identification of new varieties and their rapid increase under appropriate and generally accepted names and provision for continuous supply of comparable material by careful maintenance

In India, certification is voluntary and labeling is compulsory. Seed certification is subjected to the Seeds Act, 1966 and the procedures are regulated by the 1968 rules for Certification and subsequent amendments. Certified seed must satisfy the standards as outlined in the 2013 edition of the Indian Minimum Seed Certification Standards (IMSC). Trade in seed is subject to bi-lateral and/ or multilateral agreement at local, regional and international levels. In general, the seed trade is one of the most regulated sectors in all countries, with a plethora of seed loss, 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. Several international bodies were created for setting standards and regulations that provided an enabling environment for the seed industry: The International Seed Testing Association (ISTA); The International Plant Protection Convention (IPPC); The OECD seed schemes, and the International Union for the Protection of New Varieties of Plants (UPOV).

### **OECD Seed Scheme**

The OECD Seed Schemes provide an international framework for the certification of seed with the aim of facilitating the growth in trade of seed by reducing technical barriers (providing 'seed Passport'). The Schemes were established in 1958 in response to a combination of factors including the rapidly growing seed trade, the increase in regulatory requirements in some countries, the development of off-season production, the large breeding and production potential of exporting countries. The purpose of the OECD Seed

Scheme is to encourage the use of "quality-guaranteed" seed in participating countries. The Schemes authorize the use of labels and certificates for seed produced and processed for international trade according to agreed principles. The OECD Seed Certification Schemes are based on two key criteria; varietal identity and varietal purity. The OECD certification is applied to varieties satisfying DUS tests (Distinction, Uniformity and Stability), and the Schemes aim to ensure varietal identity and purity through seed multiplication, processing labeling. They also provide specifications for seed multiplication outside of the country, which is becoming an ever-increasing practice. In 2008, over 500 000 metric tons of seed were OECD-certified, traded and used by farmers. In addition, the main OECD principles can also be applied to seed that is used on the domestic market. There are seven distinct and independent Seed Schemes and admission to each Scheme is voluntary. India became full members of the scheme in 2009. Presently, India participates in 5 schemes namely, Cereal seed; Maize and sorghum seed; Vegetable seed; Grass and legume seed and Crucifer seed and other oil or fibre species seed.

### **Varietal maintenance**

The objective of maintenance breeding is to maintain and purify the pedigrees (parental lines) of hybrids or varieties. Thus, the genetic identity and purity of the hybrids can be preserved against various factors affecting the genetic deterioration *viz.*, genetic erosion, admixtures, selective influence of pests & diseases etc To achieve the goal of maintenance breeding *i.e.*, preserving the genes, traits, characters, hereditary factors which make one aspect of a breed or species different from another for the future. To minimize the contamination arising due to these factors, one has to take adequate care while producing seed more specifically at nucleus/breeder seed level in terms of land requirement, isolation, rouging, plant protection measures, harvesting, threshing and processing etc. The maintenance of the OECD variety is the sole responsibility of the institutes to which the variety belongs. The varietal maintenance is done through maintainer who is a person or an organisation responsible for maintaining the variety and ensuring that it remains true to type throughout its full life-span and in the case of hybrid varieties that the formula for hybridization is followed. Maintenance may be shared. For listing purposes, the maintainer can be the national office / the company/institute responsible/or the variety even when the maintenance process/or the variety takes place at another location. The maintenance of variety done by adopting the normal maintenance breeding procedures. The maintainer code is a unique alpha-numeric code attributed to each maintainer by the National Designated Authority. The list of maintainers is compiled from the individual countries' lists and comprises the names and addresses, including the country, of each maintainer.

### **Approaches for maintenance for Self-pollinating Crops**

- Produce enough breeder seed for the lifetime of the variety:

- Produce breeder seed every fifth year:
- Use basic seed as source for breeder seed without selection :
- Use Basic Seed as Source for Breeder Seed with Negative Mass Selection :
- Ear-to-row or Plant-to-row Selection for Self-pollinating Crops:

### **Approaches for maintenance for Cross-pollinating Crops**

- Negative Mass Selection :
- Ear-to-row and Plant-to-progeny Selection
- Rest Seed Method:

### **Classes and Stages of Seed Multiplication**

#### **Breeders Maintenance Material**

Checked against DUS characters. Carries Suppliers Labels. Controlled and maintained by the maintainer / breeder. Used for pre-basic seed multiplication.

#### **Pre-Basic Seed**

Carries White Label with diagonal Violet Stripe. Controlled by official certification authority (DA) and maintainer. Undertake pre- controlled test. Cannot be commercialized and not for sale. Produced officially by the recognized institute/ organization.

#### **Basic Seed**

It carries white label and is controlled by official certification authority (DA) along with the maintainer. Undertake pre-controlled test. Cannot be commercialized and not for sale. Produced officially by the recognized institute/ organization. Basic Seed shall be produced under the responsibility of the maintainer who will decide, in consultation with the Designated Authority, the number of generations from parental material before Basic Seed, which number must be strictly limited; and who will maintain a sufficient supply of seed for sowing to produce Basic Seed, ensure that it preserves the characters of the variety and supply the Designated Authority, when requested, with samples of this seed. If the Basic Seed is produced in a country other than the country of registration of the variety, technical conditions must be agreed in advance by the Designated Authorities of both countries concerned.

#### **Certified Seed**

Certified seed may be produced either inside or outside the country of registration of the variety it carries Blue Label (C 1) and Red Label (C 2). This seed is not under Breeder's / Maintainers control, however they are consulted for the number of



multiplication. Designated Authority and Controlling Authorities undertake the quality control including post control test and provision of Patent Royalty to the Maintainers / Breeder's. This class of seed is used for the commercial multiplication or sale.

### **Not Finally Certified Seed**

The seed which is to be exported from the country of production after field approval, but before final certification as basic or certified seed, shall be identified in fastened containers by the special label is referred as not finally certified seed. It carries grey label.

### **Standard Seed**

This category mainly exists in vegetable seed scheme. Seed that is declared by the supplier as being true to the variety and of satisfactory varietal purity. It must confirm to the appropriate conditions in the scheme. It carries dark yellow label.

### **Eligible Varieties and Parental Constituents**

Country shall have national list of varieties under the OECD Seed Schemes, which include only those varieties tested and listed to be Distinct, Uniform and Stable following internationally recognized guidelines and in case of agricultural species, varieties also found to have acceptable Value for Cultivation and Use (VCU) in at least one country. Registered in National Catalogue of Varieties.

### **Control of the Production of the Seed**

The Designated Authority [DA] in the country of production of the seed is responsible for implementing the Scheme in relation to that of production. For certified seed production in *OECD*, non-official inspectors are also allowed. Seed testing is done as per the International methods of Seed Testing recognized by the DA. The DA may authorize non-official laboratories to carry out seed analysis, but under official supervision.

### **The Designated Authority of the Country of Registration is responsible for**

- Ensuring that the variety to be OECD listed has been registered on the National Official Catalogue
- Communicating the name of the person(s) or organisation(s) responsible for the maintenance of the variety
- Liaising with the maintainer of the variety
- Providing written agreement for the multiplication of seed outside the Country of Registration to the appropriate Designated Authority
- Supplying an authenticated standard sample of the variety to be multiplied in order

- that a control plot can be sown to provide an authentic reference of the variety
- Supplying an official description of the variety to be multiplied, and, in the case of a hybrid variety, a description of the parental components
  - Authenticating the identity of the seed to be multiplied.

### **Requirements of the production and field inspection**

- In each participating country requirements for the production of Basic and Certified Seed approved under the Scheme as being satisfactory for varietal identity and purity shall be officially applied. These requirements shall not be lower than the standards given.
- The Designated Authority must satisfy itself by inspection of the plants at an appropriate stage or stages during production that the lot is acceptable.
- In the case of production of seed of "Certified" category, the Designated Authority may, under official supervision, authorise non-official inspectors to operate field inspection with a view to seed certification, on the conditions or applicable treat and tThe Designated Authority which decides to use this method must define the operation scope (species; territories, areas and period concerned), ensure the official check inspections, sampling and post-control tests and other requirements or described and take all necessary measures to guarantee equivalent inspection in the sense of the Schemes for field inspected by authorized inspector or by official.
- The designated Authority must take all practicable steps to ensure that the identity and varietal purity of the seed have been maintained between harvest and the fastening and labeling.

### **Seed Sampling**

The Designated Authority is authorized to take seed lot sampling, fastening and labeling of container. He may authorize non-official persons to carry out, under official supervision of seed sampling, fastening and labeling of containers. The sample shall be large enough to meet the requirement as outlined and shall be drawn according to current institutional methods for seed sampling recognized by the Designated Authority.

### **Seed Analysis**

Seed Analysis of the sample shall be carried out in the official laboratory designated by the DA for analytical purity and germination according to current International Methods for seed testing recognized by DA. The Designated Authority may authorize non-official laboratories to carry out analysis under official supervision and under such situations the DA shall undertake official check analysis and satisfy itself of verification and requirements.

### **Seed Sample Storage**

For basic seed third part of each sample shall be stored as long as possible for comparison in control plots with future test samples of Basic seed. For certified seed a third part of each sample shall be stored for one year.

### **Pre and Post Control Tests**

Pre control test is compulsory for Pre-Basic and Basic seed. A part of every sample of Basic Seed and 5 to 10 per cent of the certified seed shall be checked in a post-control test conducted immediately or in the season following the drawing of the sample

### **Issue of Certificates**

The Designated Authority may issue certificates for each lot of Pre- Basic, Basic and Certified seed approved under the Scheme for varietal purity and for analysis results according to the procedures laid out. These two certificates shall carry the same OECD reference number.

### **Blending of Lots of Same Variety / Re-Packing and Re-labeling in Another Country**

Two or more lots of certified seed of the same generation of one variety may be blended before or after export in accordance with the regulations of the country in which the seed is blended. A new reference number will be issued for blended lots. Records will be kept by the DA showing the reference numbers of the lot making up the blend and the proportion of each lot in the blend. Provision for Re-packing and Re-labeling in another country allowed.

### **Reference Numbers for Certificates and Seed Lots**

In OECD the lot numbers are assigned based on Three letter country code as per ISO-3166-1 followed by initial letters of DA followed by reference number of the lot having uniform digits (for example, 0001 to 9999) and a code letter used to indicate harvest year. The code number is given for a year.

### **Specifications for Label or Marking of Seed Containers**

Labels may be either adhesive or non-adhesive. The information may be printed on one side only or on both sides. Labels shall be rectangular in shape. The colors of the labels shall be white with diagonal violet strip for basic seed, white for certified seed, blue for first generation certified seed, red for second generation certified or successive generations. Not finally certified seed shall carry grey label. On all red labels and all grey labels for certified seed of 2nd or further generation the appropriate generation number must be stated. One end of the label shall be overprinted black for a minimum distance of 3 cm leaving the rest of the label colored.

The material used for labeling must be strong enough to prevent damage in ordinary usage. Statement of re- packing and re- labeling is given if applicable. In OECD all information shall be given in either English or French except reference to the Scheme which must be in both English and French. Label number is not given in OECD schemes.

### **Specimen Certificate and Analysis Results**

In OECD, statement of re-packing and re-labeling is given additionally. In OECD only number of containers and declared weight of the lot is given and ISTA orange certificate is given.

### **Procedure for the Extension of the Scheme**

Procedure for the extension of the scheme to include, for the purposes of field inspection, varieties under examination for registration on a National List.

### **Specific Crop Standards**

Specific crop standards shall be followed in order to meet out the varietal purity.

### **Previous Cropping**

#### **Hybrids**

Hybrid seed may not be grown in the same field for successive years.

#### **Varieties**

Grower requires furnishing particulars concerning the previous cropping in each seed field. There shall be minimum time interval at least 2 years between cereal crops of same species. Successive crops of the same variety and category of seed may be grown on the same field without any time interval provided that the satisfactory varietal purity is maintained. The growers shall furnish to the DA, particulars regarding, the previous cropping in each seed field. There shall be a minimum time interval between seed crops and any other crop of the same species as follows: for crucifer species: five years; for other species: two years.

### **Isolation**

#### **Hybrids**

For hybrids, female parent and cross pollinated OP varieties, prescribed distances from other variety of the same species except from a crop of male parent shall be maintained. Distances can be modified where there is sufficient protection from undesirable pollen or where the possibility of cross-fertilization is eliminated.

#### **Varieties**

The seed crops of self-fertilizing species shall be isolated from other cereal crops by

a definite barrier or a space sufficient to prevent mixture during harvest.

### **Weed**

Crops containing an excessive number of weeds shall be rejected.

### **Number of Harvest Years**

The Designated Authority shall decide the number of harvest years to be permitted for a seed field, with particular attention when multiplying foreign varieties to the effects of changed ecological conditions on varietal purity.

### **Field Inspection**

There shall be at least one field inspection of each seed crop after the emergence of the inflorescence. Control plots wherever possible be available for detailed examination at the time of field inspection of the seed plot. The DA shall decide whether or not approval can be given to the field following field inspections. For hybrids and parental lines minimum three inspections are followed. Official seed certification officer are authorized to inspect the plots. Non- official inspectors are also allowed to conduct inspections.

### **Varietal Purity in Seed Crop**

The varietal genetic purity varies with class of seed. For basic seed it is 99.9 per cent are certified seed 1<sup>st</sup> generation and 2<sup>nd</sup> generation 99.7. This depends upon the species and ranges from 99.9 to 95 per cent.

### **Seed Sampling (Including Fastening and Labeling of Containers) and Seed Analysis by Authorized Persons or Laboratories under Official Supervision**

The Designated Authority may authorize persons who are not under its direct and exclusive authority to draw samples under official supervision is called 'seed samplers'. Laboratories may also be authorized to carry out seed analysis as required under the Schemes. Sampling, fastening and labeling of seed containers may be entrusted to authorized persons.

### **Seed Lot Sampling**

Sampling is done by samplers and supervised by official supervisors. And five percent check sampling done by official seed samplers.

- DA may authorize non officials person to carry out seed sampling, fastening & labeling of container under official supervision (5 percent)
- DA may authorize Non-official laboratory for seed analysis.
- Model Label for container & printed information must be submitted for approval to OECD.
- White label for basic seed is not require if it is to be used in the same country.

## Seed Analysis

In OECD Seed analysis is carried out in the laboratories authorized by DA and the laboratories shall carry out seed testing in accordance with current international methods. The laboratory shall be an independent laboratory, or a laboratory belonging to a seed company. In the case of laboratory belonging to seed company, the laboratory may carry out seed testing only on seed lots produced on behalf of the seed company to which it belongs, unless it has been otherwise agreed between the seed company, the applicant for certification and the DA.

## Validity Period

No validity period is mentioned for Pre-Basic, Basic and Certified Seed.

## Off-types in Field

Maximum number of plants of the same species being not true to variety is 1 in 30sq.mt for basic seed and 1 in 10sq.mt for certified seed. In OECD standard off type plants permitted all 0.22 percent in basic seed and 0.67 percent in certified seed.

## Male Sterile Seed Parent

Male sterile seed parent may be mixed with fully fertile seed parent in the ratio of 2:1

## Down-grading Seed Class

No provision is made to the OECD seed certification in this aspect.

## Field Inspection Count

| Crop Group       | Crops                                       | OECD Seed Standards |                     |
|------------------|---|---------------------|---------------------|
|                  |   | Basic               | Certified           |
| Pulse vegetables | Pea, Cowpea, Bean                           | 100 plant per count | 100 plant per count |
| Leafy crops      | Lettuce, Spinach,<br>Coriander, Methi       | 500 plant per count | 500 plant per count |
| Fiber crops      | Jute  | 200 plant per count | 200 plant per count |
| Fruit crops      | Tomato, Brinjal , Okra,<br>Capsicum, Chilly | 100 plant per count | 100 plant per count |

## Future perspectives

The Indian Minimum Certification Standards were developed and is being adopted since 1974 for the domestic certification systems as per the need and requirement of Indian farmers and stake holders of seed industry in India. Whereas the OECD Seed Schemes Rules and Guidelines are being developed for the benefit of 58 member countries for their Varietals Certification process to meet out the International seed standards and

trading requirements. As these are two separate streams, we cannot harmonize the Indian Minimum Seed Certification Standards with the OECD Varietals Certification Rules and Guidelines. Whenever, a variety is being registered / offered for the OECD Seed Schemes then the OECD Rules and Guidelines need to be adopted and International varietal certification process has to be carried out. Similarly, the existing domestic seed certification with Indian minimum seed and field Standards will continue for the production of high quality seeds as per the Seeds Act. Both Indian Minimum Seed Certification Standards (IMSCS) and OECD Varietal Certification Systems, have agreeable level of general and specific crop standards requirements to meet the certification systems in these streams. However, certain variations have been found between IMSCS and OECD in general standards in case of: Classes and Stages of Seed Multiplication, Eligible varieties and Parental constituents, Control of the production of the seed, Seed sampling, Seed analysis, Seed sample storage, Pre- and Post Control tests, Issue of Certificates, Blending of lots of same variety, Provision of Re-packing and Re-labeling in another country, Reference numbers for certificates and seed lots, Specifications for the OECD Label or Marking of seed containers, Specimen certificate and analysis results, Procedure for the extension of the scheme, etc. The Specific crop standards namely: Previous cropping, Isolation, Off type, Varietal identification, Disease, Weed seeds, Number of harvest years, Field inspection count, Varietal purity in seed crop, Seed crop inspection, Field inspection of seed crops by Authorized Inspectors under Official supervision, Seed sampling (including Fastening and Labeling of containers) and Seed analysis by Authorized persons or laboratories under Official supervision, Validity period, Down-grading seed class, etc., are as mentioned in Table 5. In summary, we can say that even though India has become the member of OECD Seed Schemes, the two seed certification systems i.e. Indian Seed Certification and OECD Varietal Certification would move parallel in our country for the production of high quality seed as per the demand at Domestic and International trading requirements. As per the norms of OECD guidelines the Labels and Certificates designed and prepared by India as new member country shall need to be placed for the discussion and concurrence of OECD Secretariat. Multiplication of Indian Varieties in member countries for enhancing the International trading activities need to be explored by discussion with Officials of EU, ISF, ISTA, and AOSA for facilitation.

## Seed Health Testing

### Significance of seed health testing

One of the most important aspect of quality seed is the production of disease free seed, therefore, seed health testing is more important because

- Seed-borne inoculum secures the presence of a virulent strain of the pathogen along the seed.
- The presence of a pathogen with the seed favors the earliest possible establishment of the infection in seedlings.
- Un-infested soil may be inoculated by the introduction of infected seeds hence subsequent crop raised from healthy seed may in turn be infected from the soil.
- New physiological races/strains may be introduced with the seed so that varieties resistant to endemic races of the organism become affected.
- To know the health status of seed lots and keeping its quality under storage and consequently it's planting value.

### Scope of seed health testing method

The seed health testing methods have become very critical and target oriented. The methods employed for the detection of seed borne viruses and bacteria are very sensitive and accurate, however, needs fairly good knowledge of microbiology, plant biotechnology and plant pathology considering the present set up of seed analysis. Tremendous scope exists for the application of these methods.

Seed health consciousness is gaining importance. Seed health awareness is increasing. Academically more and more methods are being evolved which are accurate and less time consuming but general adoption is far away due to the involvement of scientific infrastructure, manpower and expertise in our conditions.

### Common methods employed

Techniques employed for detection of seed transmitted pathogens

The selection of a diagnostic method for evaluation of seed health depends upon the host to be tested as well as type of pathogen.

The purpose of the test are also very important.

### Conventional Techniques

Naked eye dry seed inspection,



Seed wash test,  
Whole embryo count test,  
Incubation tests,  
Grown on test,  
Infectivity test,  
X-ray radiography,  
Electron microscopy based techniques,  
Histopathological test  
Seedling symptom tests

### **Serological techniques**

ELISA (Enzyme linked immunosorbent assay) including Double Antibody Sandwich (DAS)-ELISA, Biotin-Avidin ELISA, Bead ELISA, Indirect F (ab')Z Fragments ELISA, Protein A coating (PAC) ELISA, Protein A-Sandwich ELISA, Dot-Immuno-binding Assay (DIBA), Single Antibody Dot Immunoassay (SADI), Immunosorbent Electron Microscopy (ISEM), Immunofluorescence assay, solid phase immunosorbent methods, Disperse dye-immuno Assay (DA), Enzyme linked Fluorescent Assay (ELFA).

### **Nucleic Acid Based Techniques**

Restriction Fragment Length polymorphism (RFLP) analysis, Polymerase Chain Reaction (PCR) and its different modifications such as Reverse Transcription PCR (RT-PCR), Combined Biological and Enzymatic Amplification (BIO-PCR), Taqman PCR, Immuno magnetic PCR (IMS-PCR), Multiplex PCR, Mimic PCR, Competitive PCR, Quantitative PCR, Spore-cracking PCR, Nested PCR, Repetitive sequence based PCR (rep-PCR), Reverse transcriptase PCR (PCR/RFLP), Magnetic Capture hybridization (PCR-MCH), Immunocapture RT-PCR, RT-PCR-ELISA.

### **Conventional Techniques**

#### **Inspection of dry seed**

- Pour the sample on the purity analysis board on the top left surface
- With the help of spike, separate the pure seeds into the container placed on right side below the board.
- In other two small containers, separate the other crop seed (OCS) and inert matter (IM).

- The inert matter may consists of soil, sand, stones, various types of plant debris, sclerotia, smut balls, seed galls, bunt balls of fungi.
- The physical abnormalities may appear on seed and include shriveling of the seed, reduction or increased size, discoloration or spots on the seed coat.
- Abnormal seeds/inert matter may be tested with aided light under stereoscopic microscope.
- Seeds showing abnormalities and inert matter can further be tested by blotter or agar plate method.
- Make report on all the four components (pure seed, inert matter, weed seed and other crop seed) by weight.

#### **Advantage:**

The method provides quick information on seed health and purity. Very helpful for the detection of many diseases including grain smut of sorghum and pearl millet, ergot of sorghum, pearl millet, bunt of wheat, rice and Triticale, wheat seed gall etc.

#### **Standard blotter method**

##### **Materials and testing facilities**

Petri plates (plastic or glass, 90 mm diameter), blotting (filter) papers with superior water holding capacity already cut into 90 mm diameter, distilled water, forceps; trays (glass and plastic); growth (incubation) chamber with adjustable temperature and provision of NUV light system, deep freezer (-20°C), stereoscopic binocular microscope with magnification up to 60 times, compound microscope with magnification up to 400 times, glass micro slides and cover slip, electric oven, laminar air flow system.

##### **Procedure**

- Keep the cooled pre-sterilized glass Petri plates on the clean surface of the working table in required quantity (sterilization of glassware is done in an electric oven for 2 hrs at 180°C).
- Keep the filter papers near the Petri plates, count and make the sets of 3 filter papers for one plate.
- Disinfect the forceps tips (keeping it over the flame for few seconds, cool it).
- With the help of forceps, dip one set of filter paper in a glass tray containing distilled water. After complete soaking just, lift the set in air over the tray, allowing the extra water to run-off.
- Place the moist filter paper into lower half, holding the paper with the help of forceps in right hand. Set the papers, turning the plate clockwise.
- Prepare the plates in the same way. Wipe off and dry the working label.

- Place the seeds on a plain paper sheet (Number of seeds to be plated in one plate depends on the size of the seed. In a plate 5, 10, 25 or 60 seeds can be placed). Write the accession number and date of examination of the seed sample.
- Count and make small groups of 10 or 25 seeds for one plate. Do not touch the seed use spikes.
- Arrange the counted seeds on moist blotter (lined in plates) using forceps at equidistant from each other. Close the lid (for plating the 25 seeds, keep one seed in the center, 8 in middle and 16 in outer ring. Whereas for 10 seeds, one is plated in the center and 9 in the outer ring).
- Collect the plates in the plastic trays without disturbing the seeds.
- Incubate at 20-25°C for 7 days in alternate cycles of 12 hr dark and 12 hr light (The common source of light used is the near ultra-violet (NUV) supplied by black light tubes or day light provided by cool, white, fluorescent tubes. In either case light is provided by two tubes hanging horizontally, 20 cm apart. Distance between tubes and plates should be 40 cm. Proper care for protection from NUV light must be taken wearing eye glass and hand gloves).
- After seven days of incubation, seeds are examined one by one under stereoscopic binocular microscope (associate mycoflora are identified based on habitat characters. These are also confirmed by making slides under compound microscope).
- Count the number of fungi on seeds and enter the observations in data-sheet. Also, make comments on symptoms on seed and seedlings.

### **Advantage**

Blotter method is the most convenient, cheap and efficient method. The method was first adopted by Doyer (1938). A large number of mycoflora including *Alternaria solani*, *Stemphylium solani*, *Colletotrichum dematium*, *C. lindemuthianum*, *Macrophomina phaseolina*, *Fusarium oxysporum* etc. can be detected.

### **Modifications**

#### **2, 4-D Blotter dip method**

Blotters (filter papers) are moistened with 0.1-0.2% solution of sodium salt 2, 4-dichlorophenoxy acetic acid instead of plain distilled water. Rest of the method is same.

### **Advantage**

The sodium salt retards the seed germination and seedling growth. Hence the seeds are not displaced; remain on the place where they were plated. Examination is made easy.

In 1956, Neergaard found the method very effective for the detection of *Phoma lingam* associated with cabbage (Neergaard, 1977).

### **Deep freezing blotter method**

- Seeds are plated as in blotter method.
- Incubate the plates initially for 24 hr under usual conditions in the growth chamber.
- Plates are transferred to deep freezer (-20°C) under complete darkness for 24 hr.
- Plates are retransferred to growth chamber for remaining 5 days.

### **Advantage**

Exposure of imbibed seeds on moist blotters to low temperature (-20°C) kills the seeds matter. This provides the nutrients for better development of associated mycoflora. To avoid the contamination in deep freezer method can further be modified. Blotters may be soaked in streptopencillin (0.2%) solution. The antibiotic will be effective against gram positive and negative bacteria. The method was first adopted by Limonard (1968).

### **Standard agar plate method**

#### **Materials and testing facilities**

Glassware, Petri plates, beakers, measuring cylinders, funnels, conical flasks. Chemical, agar-agar powder, sugar (sucrose or dextrose) distilled water. Equipment used are electric oven, autoclave, inoculation chamber (laminar-air flow system).

#### **Procedure**

- Prepare the potato dextrose agar medium (peeled potato slices 200 g boiled in 700 ml water, agar agar-powder 20 g dissolved in 300 ml with 20 g dextrose. Final mixing and making up volume to 1000 ml).
- Sterilize the media in autoclave (121.6°C for 20 min at 15 lb-pressure) and after semi-cooling, pour the melted medium in pre sterilized Petri plates (approximately 17-20 ml per 90 mm Petri plate) under aseptic conditions of laminar flow.
- After solidification of medium, invert the plate for 12 hr.
- Reject the contaminated plates (contamination refers to the development of bacterial, fungal, actinomycete or mix colonies on the medium. This also indicates the improper sterilization of glassware or media or preparation faults).
- Place 10 surface sterilized seeds (treated with NaOCl) on the media under aseptic conditions. One in center and 9 in outer ring.
- Incubate the plates containing seeds in the growth-chamber for 7 days.
- Examine the seeds for the developing associated mycoflora by naked eye and stereoscopic binocular on the basis of habitat characters.

### **Advantage**

Method is most suitable for the detection of internally seedborne fungi e.g. species of *Ascochyta*, *Macrophomina*, *Phoma*. Muskett and Malone (1941) first used the method. The method can be modified in various ways either by changing basic media, its composition or addition of chemicals, as per need.

### **Modifications**

#### **Peptone-PCNB method**

The basic medium (PDA) can be replaced by Malt-agar or Peptone-agar. Medium is supplemented by antibiotics (e.g. chloro-tetracycline, streptomycin sulphate) and / or fungicide (PCNB penta-chloro-nitro-benzene) in different concentrations.

### **Advantage**

Medium containing antibiotics and fungicides needs no sterilization. The medium is advantageous for the detection of *Fusarium* spp., *Macrophomina phaseolina* (Limonard, 1968). Hag Borg *et al.* (1950) first used 2, 4-D in agar plate for *Colletotrichum lindemuthianum* on bean seed. Other selective medium includes e.g. Oxgall-PDA medium for *Septoria* sp., Guaiacol agar medium for *Pyricularia*.

#### **Rolled paper towel method**

- Take two sheets of standard germination testing paper (paper towel); enter the number, date and crop on the other side with waterproof ink.
- Mist first sheet with sterile-distilled water and stretch over clean surface of working table.
- Arrange 50 seeds in 5 rows of 10 seed at equidistant as in germination test on one sheet. Total 400 seeds are placed.
- Cover the seeds by second pre-soaked sheet carefully without disturbing the already arranged seeds.
- Roll and tie the sheets with rubber band at both the ends.
- To avoid water losses, use butter wax coated paper for rapping the sheets at one side.
- Place the paper towels, containing seeds in germinator with slightly tilting the bunch of towel.
- Incubate in dark.
- Observe the towels after 7-14 days by opening and removing the cover sheet.
- Examine the seeds by naked eye and stereo binocular for the presence of mycoflora and seed germination.

### **Advantage**

The method is most suited for the detection of *Fusarium* spp. in cereals and *Ascochyta* spp. in pea. It is equally good for testing germination of seeds.

### **Seed washing test**

- Take one gram of seed from a working sample in a clean and small conical flask.
- Add 10 ml of water and a drop of wetting agent (e.g. Tween 20).
- Make 10 replications
- Shake the flasks for 10 minutes with care.
- Transfer the water in tubes and centrifuge for 10 minutes at 2300-2500 rpm.
- Decant the supernatant liquid leaving the sediment at the bottom of the tube.
- Suspend the sediment in 2 ml of distilled water.
- Examine the water drops under compound microscope (200 x) for the presence for oospores. The oospores are yellow-brown, spherical, 3 layered thick walled spores.

### **Advantage**

The method is best suited for the detection of downy mildew of sunflower (*Plasmopara halstedii*) and pearl millet (*Sclerospora graminicola*) pathogen. The viability of oospores associated with seeds can be determined. Oospores are subject to TTC (2,3,5 tri phenyl tetrazolium chloride) solution (1%) for 48 hr at 30°C. In complete darkness. Viable spore shows red color in their cytoplasm. The method has been used by Chahal *et al.* (1994) and Shetty *et al.* (1978).

### **Test tube water agar seedling symptom test**

- Take clean, rimless glass test tubes of 16 mm diameter.
- Dissolve 10 g agar agar-powder in 1000 ml distilled water and autoclave at 15 lb for 15 min.
- Transfer 10 ml of water agar into each tube under aseptic condition.
- After solidification of agar place one seed in each tube. Cover the mouth of tube with a piece of aluminum foil.
- The tubes are placed vertically in a tray and incubated in growth chamber as in standard blotter method.
- As the seedling reaches the cover, the foil is removed.
- Symptoms caused by the associated mycoflora on the seed and developing seedling are observed after 14th day.

### **Advantage**

The method is suited for most of the pathogen. Symptoms can be seen on roots and shoot portion. The method is also useful for quarantine stations as diseased seedlings of valuable crop can be destroyed. Healthy seedlings can be retained and saved. The method was developed for *Septoria nodorum* associated with wheat seeds (Khare *et al.*, 1977).

### **Sodium hydroxide seed soak method**

- Dissolve 2 g NaOH in 1000 ml (for preparing 0.2% solution).
- Working seed sample consists of 4000 seed with 2 replication of 2000 seed each for foundation seed and 800 seeds with 2 replications of 400 each seed for certified wheat seed is prepared.
- Seeds are soaked in a small conical flask or beaker of 250 ml capacity for 24 hr at 25°C.
- A solution is decanted after 24 hr and washes the seeds in tap water.
- Seeds are placed on blotter to soak extra water.
- Spread the soaked seeds over white background and examine by naked eyes.
- Seeds exhibiting jet black shiny appearance with hollow or without hollowness are separated.
- Number of such seeds are counted as infected seeds and reported in percentage.
- Black seeds can be observed under compound microscope for the presence of teliospores.

### **Advantage**

The method is well suited for Bunt of wheat and Triticale (*Neovossia indica*), rice (*Neovossia horrida*) (Agarwal and Verma (1983); Sharma and Agarwal (1996); Agarwal and Shrivastava (1981); Savitri and Sattar (1996). The method is very cheap and convenient. The method can be tested even for the seeds treated with (colored) fungicides (Agarwal and Mathur, 1992). Sodium hydroxide removes the other coatings, stain, dust; chemicals etc. from the seed and cause a bit swelling of the seed to exhibit clear symptom of the disease even in minute cases of infection.

### **Embryo count method**

Embryo count method for the detection of loose smut infection in wheat seed is described by Agarwal *et al.* (1978); Gaur and Agarwal (1995). A cost effective suitable and modified technique is presented by Singh and Maheshwari (1995) consisting of the following steps.

## Materials and testing facilities

Glassware/plastic wares; beakers, Petri plates, glass rod, plastic bucket, mugs, glass funnel attached with tube and stopper measuring cylinders. Chemicals; sodium hydroxide, trypan blue, rectified spirit, phenol, glycerol, lactic acid, Equipment; stereoscopic binocular microscope, precision balance, counter general; sieve mesh 10, 20 and 30 size, cheese cloth, seed sample, hot plate tap water system.

## Procedure

- Soak 100 g (2200-2800 seeds) wheat seeds in 100 ml of 5 per cent solution of sodium hydroxide (NaOH) and 0.005 per cent trypan blue in a 2000 ml capacity beaker for 18-22 hr at 22-24°C.
- Pass the soaked seeds through a sieve set of 10 and 30 mesh and wash the seeds thoroughly with running tap water to separate the embryos.
- Agitate the soaked seed material to facilitate the separation of embryos. Collect the embryos over 30 mesh size.
- Wash the separated embryos into a tea strainer and dehydrate them with methyl spirit for 2-5 minutes.
- Pass the dehydrated embryos in solution of lactic acid + glycerol + water (1:2:1) in small beaker.
- Take a glass funnel; connect the stem of the funnel with rubber tubing provided with a stopper.
- Pass the mixture of lactic acid + glycerol + water through the tunnel. The embryo float at the top of the funnel and chaff sinks.
- The chaff can be run-off through a tea strainer and collect the solution in a beaker which can be reused for extracting the embryos for additional 3 samples.
- Repeat the process 4-5 times until embryos are separated from chaff. Heat the solution with embryos for 2 minutes until boiling.
- Allow the solution to cool for 30 minutes.
- Arrange the embryos in Petri plates in ring of individual embryo and pour solution of lactic acid and glycerol to facilitate examination.
- Evaluate the test by examining less than 12 to 15 x magnifications under stereoscopic binocular microscope. Infected embryo exhibit blue stained mycelium of loose smut pathogen in scutellum, plumule or whole embryo.

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## Seed Quality Assurance and Enhancement: Scope and Opportunities

The development of new plant varieties / hybrids, having desirable attributes with respect to productivity, tolerance to biotic and abiotic stresses or special quality traits, not only demands high intellectual input, but also requires considerable time and investment. Good quality seed is the vehicle to carry the advantages of such varieties to the end users. Hence, the quality status of seed is of critical importance to the seed producers as well as the farmers. Though the crucial role of seed in the whole process of agriculture are well recognised since the evolution of agriculture, the parameters of seed quality were determined only about a century ago and a system of quality evaluation was developed to meet the demands of modern agriculture. The precision of measurement and reproducibility of various tests have been tested and standardized over several decades and still being continuously upgraded. The advancements and refinements in seed testing procedures brought in the last two decades have been as significant, as the technological innovations and inventions seen in plant breeding itself.

In the initial years, quality seed production only comprised of simple steps, such as selecting the best plants for seed collection, harvesting, threshing and cleaning the seeds from such plants separately, drying, cleaning and storing them in cool and dry conditions, using various locally available innovations, which resulted in better seed quality. It is only after the spread of seed trade as an agri-business, that an organized system of quality assurance came into practice. Since the establishment of the first Official Seed Testing Laboratory in Tharandt, Germany in 1869 by Professor Friedrich Nobbe, the seed quality assurance system in general, and seed testing in particular, have come a long way. Three factors were responsible for this:

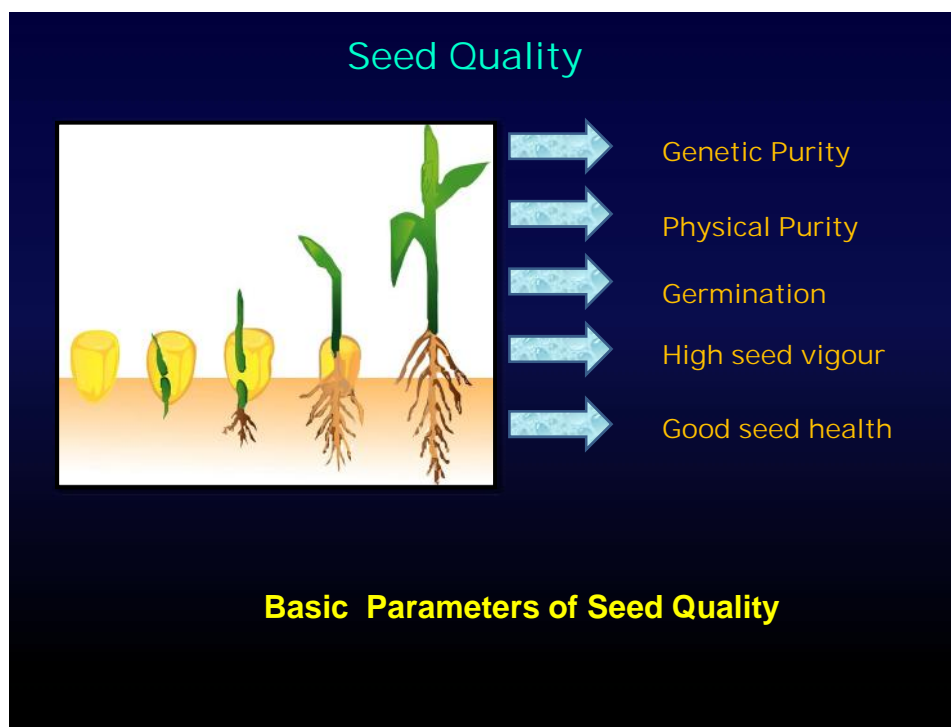
- growing awareness about the quality of seed in the success of agriculture
- large scale seed trading between and within the farmers of different communities, regions and countries and
- introduction of policies, acts and regulations by different governments to support agriculture recognizing its vital role in a country's economy

In India, the introduction and enforcement of the Seeds Act, 1966 in 1969, coincided well with the era of Green Revolution. The Act provided for a formal system of seed quality control in the country to ensure that the advantages of new improved varieties reach the farmers, though the informal system of quality assurance was prevailing since the beginning of the twentieth century and followed in some form or the other by some of the private seed producing companies, mostly on the lines of Emergency Seeds Order of 1917 and the Seeds Act, 1920 of the UK. It is relevant to point out that as per the Seeds Act, 1966

all seeds sold in the market must be labeled and any seed of a notified variety/kind sold in the market must meet the minimum criteria of physical purity, germination and moisture. In case of certified seed, the testing is conducted and validated by a Seed Certification Agency, whereas in case of non-certified or labeled seed, the purchaser relies on the assurance by the producer, though the testing could have been made in a notified seed testing lab or a private laboratory. Similarly, the authenticity and purity of the variety are also based on the assurance of the producer company. Though the three main components i.e. varietal purity, germination and physical purity remain the core components of seed quality, several additional parameters viz., seed health or presence of seed borne/transmitted pests and pathogens; vigour (planting value) of the seed, variety purity and trait purity in GM varieties are also evaluated through special tests to ensure the highest planting value of a seed lot.

### 1. Components of Seed quality assurance

The system of seed quality assurance relies on the testing of four primary components of seed quality, i.e. genetic (variety) purity, physical purity, germination and moisture. Testing for the seed borne pathogen is required in case of designated diseases.



Evaluation of seed quality can be made at four levels of testing:

- Field inspection of the seed crop and quality evaluation of the processed seed by the seed producing organization itself

- Field inspection and seed testing performed by the designated Seed Certification/Seed Testing agencies
- Market sample collection by Seed Inspectors and its testing in a designated laboratory and
- Quality check of a purchased seed lot by the farmer before its use (normally applies to the seed lots procured/purchased from less reliable sources).

The first two of the above constitute the basic mechanism for a reliable quality control system. However, there are several types of formal, official, quasi-official and voluntary seed quality assurance mechanism operative globally. Most of the countries have an official system of Seed Testing in place, which is operated as per the country's legislation by the respective designated laboratories. For following a uniform system of seed testing, the International Seed Testing Association (ISTA) developed standardised protocols of testing various quality parameters. The working Groups of ISTA, in collaboration with the ISTA member laboratories, undertake regular revisions of protocols and standardization of new protocols for existing or new parameters. Some of the newly added components of seed quality, for which protocols have been standardized more recently for some crops and being standardized for more, are:

- (i) Vigour assessment methods
- (ii) Molecular techniques for species and variety determinations and genetic purity testing
- (iii) ELISA and PCR based techniques for identification of seed-borne pathogens
- (iv) Sampling and detection protocols for determining the adventitious presence of GM seed
- (v) Sampling and quantitative determination of trait purity in GM varieties

Of these, vigour testing by Accelerated Ageing / Controlled Deterioration test or Conductance of seed leachate (for pea, soybean and Brassicas, more species are being added), electrophoresis and iso-electrophoresis methods for hybrid purity of maize, sunflower and Immunological/DNA based detection methods for seed transmitted virus and bacterial pathogens have already been included in the ISTA's recommended methods.

Presently there are 108 notified Seed testing Laboratories in the country, though a majority of these are equipped only for performing the primary seed quality testing, i.e., physical purity, germination and moisture determination. The Govt. of India has taken several steps to strengthen the STLs in terms of physical infrastructure and training the technical staff to improve the level of their functioning. Internationally, it is mandatory that a Seed Analyst must have necessary technical competence as an essential qualification. The analyst may either hold a degree or diploma or certificate in Seed Technology/Seed Testing

to be eligible for such position. It is also desirable that the analysts regularly undergo refresher courses and upgrade their competence in view of the technological advances which ensure higher levels of precision in results.

In addition to the Govt. notified STLs, several of the Seed Testing Laboratories in the private seed sector have obtained the ISTA accreditation for different components of seed testing and issuance of certificates required for the purpose of international seed trade. Thus, these laboratories are recognized for quality assurance internationally. Reliability of seed testing largely depends on methodologies/protocols followed, competence of the analysts and necessary infrastructure for operational accuracy. Inadequacy in any of the above, leads to discrepancies in the results of the testing performed by the Seed Testing Laboratories.

In addition, a number of privately owned organizations companies in many countries provide services for seed quality assurance or testing different parameters of seed quality using immune-based, DNA-based, protein profiling, chromatographic or bio assay based techniques for evaluating seed purity, health, herbicide tolerance, Bt/transgene etc. Australian organizations such as "Rural Solutions" and "Sure Seed" offer seed services to their clients and are accredited to issue OECD, AOSCA or ISTA certificates, following their prescribed testing programmes. Several ISO certified laboratories also provide similar services to the seed producers. Many countries viz. USA, Canada, Australia recognize the seed testing laboratories in the private seed companies for the purpose of quality assurance, though in India, the notified seed testing laboratories are operated only by the government organizations. However, for the purpose of labelling, the seed testing can be performed in the laboratories in the private or public sector organization.

## **2. Uniformity, Accuracy and Reproducibility**

In order to achieve precision in testing, the International Seed Testing Association, Zurich publishes a set of internationally validated and accepted procedures (Rules) for performing each of the seed quality tests for a large number of plant species including field crops, horticultural species, trees, shrubs, herbs and medicinal species etc. Most of the countries have adopted ISTA procedures for developing the national seed testing manuals, including India. North Americas follow a set of testing procedures and rules, very similar to those of ISTA, but validated and prescribed by the AOSCA. In order to achieve uniformity in (a) tests repeated on the same seed lot by the same analyst, (b) tests performed for different samples of the same lot by the same analyst, (c) tests performed on the same sample by different analysts or (d) tests performed on the samples of the same lot in different laboratories, it is important that all participating laboratories follows the same procedures and participate in the "ring tests" or "proficiency tests" coordinated by the



nodal centre. All State Seed Testing Laboratories (SSTL) are meant to analyse seed samples of any notified kind or variety received from any source for the purpose of

- Analysis of samples Under Section 8 of the Seeds Act (received from SCA).
- Seed labeling, selling or using for cultivation.
- Compliance of labeling Under Section 7 of the Seeds Act (received from Seed Inspectors).

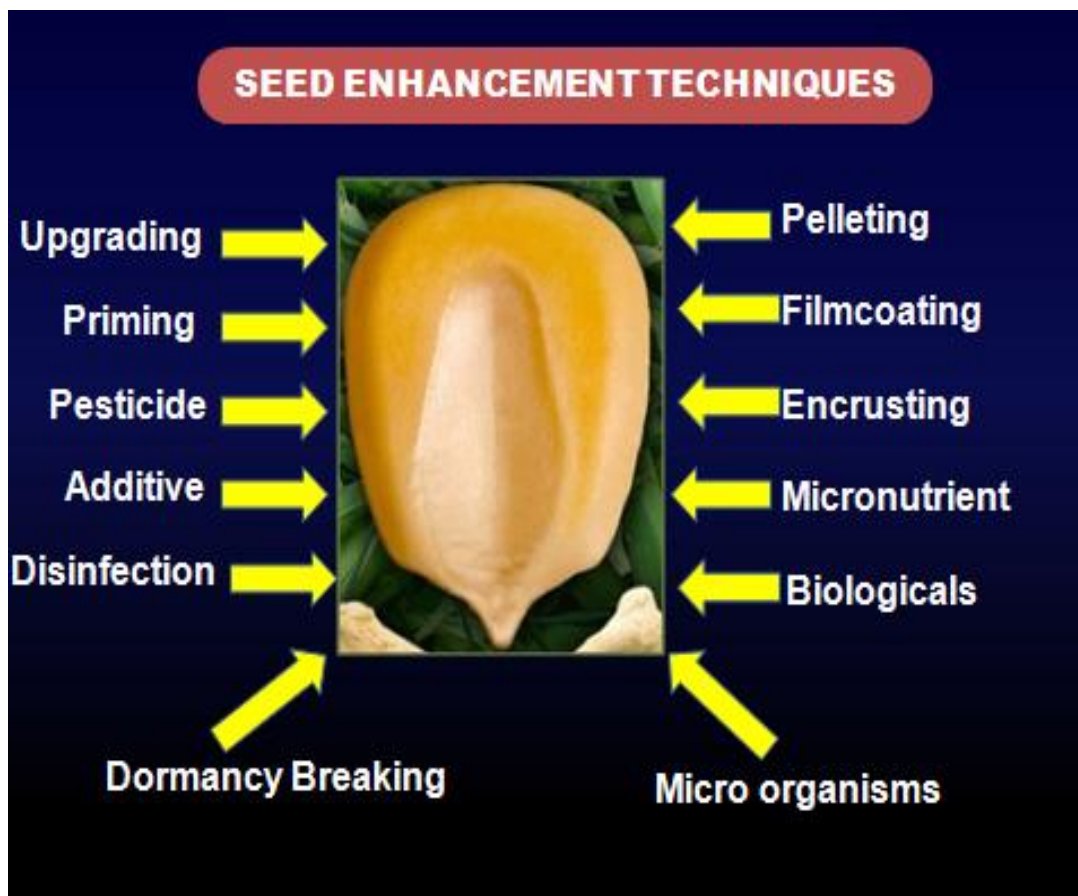
In India, the Central Seed Testing Association, NSRTC, run by the DOAC, Ministry of Agriculture is the coordinating unit, mandated with the responsibility of a Referral Laboratory. It receives at least 5% of all seed samples received by the notified Seed Testing Laboratories and validates the accuracy of test results. It also conducts annual workshops and organizes training for updating the competence of the analysts working in different STLs.

Statistical Tools for ST: Application of statistical methods for accuracy of various test results is another important component of quality assurance. ISTA recommended Tolerance Tables are applied to validate the accuracy of test results within or between different laboratories and lots. Modern tools and softwares are being developed to assist seed analyst to derive reliable inferences. Seedcalc 8 (Microsoft Excel application written for Window 2000 and Xp) for testing purity/impurity including GM traits; statistical packages for inter laboratory tests using ISO 5725; Heterogeneity Testing Calculator for seed lots in multiple containers, are some of the new introductions for Seed Quality Assurance by international bodies viz. ISTA, AOSA etc.

### **3. Seed Quality Enhancement**

The vigour and germinability (performance potential) of a seed reach highest level at physiological maturity. A seed lot, which is a population of seeds comprise of one harvest which undergoes continuous and gradual decline from harvest till complete loss of viability. As the field conditions at the time of sowing are not always optimal for germination and seedling growth, to ensure highest planting value of a seed lot, various seed treatments are employed to boost the seed quality further. Even though the seed deterioration is inevitable, its rate (the speed at which deterioration takes place) could be slowed down through different technological interventions like seed conditioning, seed processing, super drying, seed packing and controlled atmospheric storage. Similarly, the vigour of low and medium vigour seeds could be enhanced through a range of techniques like seed priming, seed coating, pelleting and treatments with pesticides and other additives. The progress in such technologies in last five decades has led to the emergence of a new sub branch under seed technology, broadly termed as "Seed Quality Enhancement".

Seed Invigoration treatments, physical, physiological, chemical, biological or a combination, that helps in boosting the vigour of a seed lot to a perceptible level upon storage or sowing under favourable or unfavourable growing conditions opened a new branch of seed research. The technologies like seed conditioning or processing, seed protection (seed treatments with pesticides), physiological enhancement of seeds (priming), seed coating/pelleting and several modifications of these are collectively examined under this new branch.



The recent advances in biological and engineering sciences has led to the invention and application of several new technologies for enhancing the quality of the seeds. Such second generation seed treatment technologies will also be critical growth drivers of seed industry in the coming years. Some of the commonly practiced technologies / methods of quality enhancement are briefly discussed below.

- 1.1 Seed Priming: Seed priming is defined as the controlled uptake of water to initiate the early events of germination, but not sufficient to permit radicle protrusion, followed by drying" McDonald (1999). The metabolic advancements of seed

through regulated / controlled hydration, is the most common method of seed invigoration which can be modified in several ways, some of which are low temperature hydro priming; osmopriming; drum priming; matrix priming; mid-storage short soaking priming etc. The conditions and duration of hydration and post priming dehydration are critical considerations. A modification of the same, Halopriming, which could be useful in hardening of seeds particularly for saline conditions, can be applied by adding various salts in very low concentrations (typically 0.001- 0.0001M) during hydration of seed.



Improvement of Stand Establishment by Seed Priming

- 1.2 Seed Coating and Pellinging : Coating the surface of the seed and turning it into a regular shaped pellet by using natural or synthetic substances are practiced not only to improve the physical and handling properties of seed, but also to use it as a means to deliver various active ingredients useful in boosting the performance of the germinating seeds. The materials used for this can be as simple as clay or riverbed silt, leaf powders, natural gums and polymers or highly advanced sensor based patented polymers such as "Intellicoat", developed for coating seeds with Intelimer® polymers, which differ from other polymers in that they can be customized to abruptly change their physical characteristics, when exposed to high or low temperatures through a pre-set temperature switch.
- 1.3 Treatment with Pesticides : Field emergence and stand establishment are greatly affected by the soil-borne pests and pathogens, particularly when the vigour is low. It is therefore, recommended that to boost the vigour of the emerging seedlings, pesticidal treatments are incorporated. Pesticides can be incorporated either directly (seed dressing) or through a delivery medium viz., polymer coating.
- 1.4 Treatment with bioactive substances: A range of materials including microbial cultures, growth regulators, natural by-products (viz., cowdung / cow urine etc.)

and vitamins are used as seed treatment for boosting its planting value under specific field conditions.

- 1.5 Treatment with nutrients: Deficiency of some micro and macro nutrients in the soil can severely affect the early vegetative growth in the field. To address this treating seeds with very low doses of inorganic compounds are found effective.
- 1.6 Physical treatments to upgrade seed quality: There is a range of physical stimulants and treatments which improve the planting value of seeds either by enhancing the latent positive energy levels (viz., EM/RF/MW ) or by countering the negative / deteriorative energies (viz. Free Radicals), by controlling the seed borne/transmitted pathogens and insects etc. Various kinds of magnetic and electro magnetic treatments are known to stimulate plant metabolic activities. Many of such technologies have been applied for increasing the storage life of biological products. Heat treatments viz., Flash Heat, Hot Water, Hot Vapour and Microwave treatments are applied for pest control as well as dormancy release purposes. Electromagnetic and radio frequency treatments have shown 5-10% increase in germination and significant enhancement in speed of germination and early seedling growth, whereas, microwave treatments were effective in control of pests and safe release of hard seededness in a number of plant species. Cathodic protection and electron dense treatments have been found effective for seed longevity.

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## Seed Testing: Sampling And Physical Purity Analysis

Seed testing is essentially done to determine the quality of the seed and it provides information on quality factors of seeds for the seed sellers and farmers. Seed quality evaluation is done in the seed testing laboratories and they play a vital role in the seed certification. It is done to obtain accurate and reproducible results in respect of physical purity, moisture, germination and other distinguishable variety (ODV). Seed testing is the science of evaluating the planting value of seeds that has been developed to achieve certain objectives for minimizing the risks of planting low quality seeds. It is the **'hub'** of seed improvement programs.

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.

### Sampling in Seed Testing

The object of sampling is to obtain 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.

#### 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 missing 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 subsample thereof. The submitted sample may be divided into subsamples 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 subsample thereof, on which one of the quality tests described in the 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 is 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 that 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 belong. All containers of a seed lot must be marked with same unique seed lot designation (numbers, characters or combination of both). Marking of samples and subsamples must ensure that there is always an unambiguous link between the seed lot and the samples and subsamples.

**Treated seed:** "Seed treatment" is a generic term which indicates that a seed lot has been subjected to:

- The application of a compound including chemicals, nutrients or hormones
- The application of a biological product including micro-organisms
- A process including wetting and drying
- Energy treatment including heat, radiation, electricity or magnetism but does not specify the application method.

**Coated seeds:** Coated seeds are seeds covered with material that may contain pesticides, fungicides, dyes or other additives. The following types of coated seeds are defined:

**Seed pellets:** More or less spherical units, usually incorporating a single seed with the size and shape of the seed no longer readily evident.

**Encrusted seed:** Units more or less retaining the shape of the seed with the size and weight changed to a measurable extent.

**Seed granules:** Units more or less cylindrical, including types with more than one seed per granule.

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

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

**General principles of sampling:** Under seed law enforcement programme only trained and experienced officials are authorized to undertake sampling and he has to give notice to such intention to the person from whom he intends to take sample.

**Method of obtaining primary samples from bulk:** The numbers of bags are checked in each lot, and numbers of samples are drawn using an appropriate sampler/trier. Samples such drawn are emptied in a clean bucket type container, till the required quantity (no. of samples) is obtained. These are mixed well and transferred to a clean polyethylene bag and tied with a label furnishing necessary information. Take three representative samples in the prescribed manner, mark and seal.

1. One sample to be delivered to the person from whom it has been take.
2. Second to be sent for analysis to the Seed Analyst of the area.
3. Third to be retained for any legal proceedings.

At least two persons should be present and obtain signature of both witnesses on Form VIII of the Seed Rules. Sampler must verify the information provided on the label as per the requirements of the Seed Act.

Following information should be checked on label:

- Kind
- Variety
- Lot number
- Date of test
- Seller's name & address.

In case of certified lots sampler should check the following information:

- Information on seed certification tag
- Name and 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.

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

**Sampling intensity:** For seed lots in containers of 15 kg to 100 kg capacity (inclusively), the sampling intensity according to Table 1 shall be regarded as the minimum requirement.

Table1. Minimum sampling intensity for seed lots in containers of 15 kg to 100 kg capacity.

| Number of containers | Minimum number of primary samples to be taken |
|----------------------|---|
| 1-4                  | 3 primary samples from each container         |
| 5-8                  | 2 primary samples from each container         |
| 9-15                 | 1 primary samples from each container         |
| 16-30                | 15 primary samples from the seed lot          |
| 31-59                | 20 primary samples from the seed lot          |
| 60 or more           | 30 primary samples from the seed lot          |

Table 2. Minimum sampling intensity for seed lots in containers of more than 100 kg, or from streams of seed entering containers.

| Number of containers | Number of primary samples to be taken |
|----------------------|---------------------------------------|
| Up to 500 kg         | At least five primary samples         |



|                  |  |
|------------------|--|
| 501-3000 kg      | One primary sample for each 300 kg, but not less than five |
| 3001-20000 kg    | One primary sample for each 500 kg, but not less than 10   |
| 20001 kg & above | One primary sample for each 700 kg, but not less than 40   |

### Equipments for sampling

- **Sleeve or stick:** The tube and sleeve have open slots in their walls so that when the tube is turned until the slots in tube and sleeve are in line. Seed can flow into the cavity of the tube, and when the tube is given half turn the openings are closed.
- **Bin trier:** It is larger than sleeve trier constructed on the same principle. It is used for sampling from heaps and godowns.
- **Nobbe trier:** Pointed tube long enough to reach the center of the bag with an oval hole near the pointed end.

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

**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 seeds falls by gravity over the cone where it is evenly distributed to the channels and spaces, then passes through the spouts into the seeds pans. It is a large divider, designed for large seeds and grains.

**Soil divider:** It is simple divider, built on the same principle as the conical divider, is the soil divider. The channels are here arranged in a straight row instead of a circle as in the conical divider. The soil divider consists of a hopper with attached channels or ducts, a frame to hold the hopper, two receiving pans and a pouring pan. In using the divider the seed is scattered fairly evenly in a pouring pan the length of the hopper and poured in approximately equal rates along the entire length of the hopper. The divider is suitable for large-seeded and chaffy species, but suitable type for small seeded species can also be made.

**Centrifugal divider:** The centrifugal divider (Gamete type) makes use of centrifugal force to mix and scatter the seeds over the dividing surface. In this divider the seed flows downward through a hopper onto a shallow rubber cup or spinner. Upon rotation of the

spinner by an electric motor the seeds are thrown out by centrifugal force and fall downward. The circle or area where the seeds fall is equally divided into two parts by a stationary baffle so that approximately half the seeds fall in one spout and half in the other spout. The centrifugal divider tends to give variable results when not carefully operated.

**Rotary divider:** The rotary divider comprises a rotating crown unit with 6 to 10 attached subsample containers, a vibration chute and a hopper. In using the divider the seed is poured into the hopper and the rotary divider is switched on so that the crown unit with the containers rotates with approximately 100 rpm and the vibration chute starts to feed the seed into the inlet cylinder of the rotating crown. It divides the seed stream into a lot of subsamples.

**Variable sample divider:** The variable sample divider consists of a pouring hopper and a tube that rotates about 40 revolutions per minute. In this divider, the position of the two hoppers in relation to each other can be adjusted accurately, resulting in pre-determined subsample sizes.

**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.

**Spoon method:** Useful for samples of a single small-seeded species and also for sample reduction for moisture determination or seed health testing. A tray, a spatula and a spoon with a straight edge are required. After preliminary mixing, pour the seed evenly over the tray; do not shake the tray thereafter. With the spoon in one hand, the spatula in the other, and using both, remove small portions of seed from not less than five random places. Sufficient portions of seed are taken to constitute a sub-sample of approximately, but not less than, the required size.

**Hand halving method:** In the International Rules this method is the most satisfactory method for chaffy and genera of tree and shrub seeds. The hand halving method can also be used with the species where all other dividing methods are extremely difficult or impossible to use.

### Lot and Sample weight of important cereal crops

| Crop         | Max. wt. of Seed lot (kg) | Minimum wt. of sample (g) |         |
|--------------|---------------------------|---------------------------|---------|
|              |                           | Submitted                 | Working |
| Paddy        | 20,000                    | 400                       | 40      |
| Wheat        | 20,000                    | 1,000                     | 120     |
| Barley       | 20,000                    | 1,000                     | 120     |
| Pearl millet | 10,000                    | 150                       | 15      |
| Sorghum      | 10,000                    | 900                       | 90      |
| Maize        | 40,000                    | 1,000                     | 900     |
| oats         | 20,000                    | 1,000                     | 120     |

### Lot and Sample weights of important pulses crops

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

### Lot and Sample weights of important oil seed & fiber crops

| Crop                | Max. wt. of Seed lot (kg) | Minimum wt. of sample (g) |         |
|---------------------|---------------------------|---------------------------|---------|
|                     |                           | Submitted                 | Working |
| Groundnut (pods)    | 20,000                    | 1,000                     | 1,000   |
| Groundnut kernels   | 20,000                    | 1,000                     | 600     |
| Mustard             | 10,000                    | 160                       | 16      |
| Soybean             | 20,000                    | 1,000                     | 500     |
| Sunflower varieties | 20,000                    | 1,000                     | 250     |
| Sunflower Hybrid    | 20,000                    | 250                       | 125     |
| Safflower           | 10,000                    | 160                       | 16      |
| Cotton              | 20,000                    | 1,000                     | 350     |
| Jute                | 10,000                    | 150                       | 15      |

### Lot and Sample weights of important vegetable crops

| Crop            | Max. wt. of Seed lot (kg) | Minimum wt. of sample (g) |         |
|-----------------|---------------------------|---------------------------|---------|
|                 |                           | Submitted                 | Working |
| French bean     | 20,000                    | 1,000                     | 700     |
| Bottle gourd    | 20,000                    | 700                       | 70      |
| Cucumber        | 10,000                    | 150                       | 70      |
| Tomato          | 10,000                    | 70                        | 7       |
| Tomato (hybrid) | 10,000                    | 7                         | 7       |
| Onion           | 10,000                    | 80                        | 80      |
| Lettuce         | 10,000                    | 30                        | 3       |
| Carrot          | 10,000                    | 30                        | 3       |
| Cabbage         | 10,000                    | 100                       | 10      |
| Okra            | 20,000                    | 100                       | 140     |
| Brinjal         | 10,000                    | 150                       | 15      |
| Cauliflower     | 10,000                    | 100                       | 10      |
| Radish          | 10,000                    | 300                       | 30      |
| Spinach         | 10,000                    | 500                       | 50      |
| Methi           | 10,000                    | 40                        | 4       |

### Conditions for Issuing Orange International Seed Lot Certificates

The sampling methods laid down in the ISTA Rules shall be followed when seed samples are drawn for the issue of Orange International Seed Lot Certificates. Further conditions have to be fulfilled as per ISTA rules with respect to Seed lot size, marking/labeling and sealing of containers, method of sampling from the seed lot, submitted sample size for various tests, sample reduction methods and storage of submitted samples after testing.

### Heterogeneity testing for seed lots in multiple containers

The object of heterogeneity testing is to detect the presence of heterogeneity which makes the seed lot technically unacceptable for sampling according to the object of sampling for testing.

**The H-Value test:** The testing of predominantly in-range heterogeneity of an attribute adopted as an indicator involves a comparison between the observed variance and the acceptable variance of that attribute. The container-samples of a seed lot, are samples drawn independently of each other from different containers. The examinations of containers-samples for indicating the attribute must also be mutually independent. Since there is only one source of information for each container, heterogeneity within containers is not directly involved. The acceptable variance is calculated by multiplying the theoretical

variance caused by random variation with a factor for additional variation, taking into account the level of heterogeneity which is achievable in good seed production practice. The theoretical variance can be calculated from the respective probability distributions, which is the binomial distribution in the case of purity and germination, and the Poisson distribution in the case of the other seed count.

**Table.** Sampling Intensity and Critical H-Values: Number of independent container samples to be drawn as depending on the number of containers in the lot and critical H-values for seed lot heterogeneity at a significance level of 1% probability.

| Number of containers in the lot | Number of independent container samples | Critical H-value for purity and germination attributes |              | Critical H-value for other seed count attributes |              |
|---------------------------------|---|--|--------------|--|--------------|
|                                 |   | Non-chaffy seeds                                       | Chaffy seeds | Non-chaffy seeds                                 | Chaffy seeds |
| 5                               | 5                                       | 2.55   | 2.78         | 3.25   | 5.10         |
| 6                               | 6                                       | 2.22   | 2.42         | 2.83   | 4.44         |
| 7                               | 7                                       | 1.98   | 2.17         | 2.52   | 3.98         |
| 8                               | 8                                       | 1.80   | 1.97         | 2.30   | 3.61         |
| 9                               | 9                                       | 1.66   | 1.81         | 2.11   | 3.32         |
| 10                              | 10                                      | 1.55   | 1.69         | 1.97   | 3.10         |
| 11-15                           | 11                                      | 1.45   | 1.58         | 1.85   | 2.90         |
| 16-25                           | 15                                      | 1.19   | 1.31         | 1.51   | 2.40         |
| 26-35                           | 17                                      | 1.10   | 1.20         | 1.40   | 2.20         |
| 36-49                           | 18                                      | 1.07   | 1.16         | 1.36   | 2.13         |
| 50 or more                      | 20                                      | 0.99   | 1.09         | 1.26   | 2.00         |

### The Purity Analysis:

The object of the purity analysis is to determine:

- The percentage composition by weight of the sample being tested and by inference the composition of the seed lot
- The identity of the various species of seeds and inert particles constituting the sample.

It ensures that a seed lot meant to be use for growing does not contain seeds/materials other than the desired kind and variety, beyond permissible limits. It helps in:

1. Improving the plan stand (by increasing the pure seed component).
2. Raising a uniform and true to type crop (by eliminating other crop & weed seeds).

3. Raising a disease free-crop (by eliminating inert and disease causing matter).
4. Mechanization and precision (by farming removing undesirable particles).

Physical purity analysis is a pre-requisite for:

- Seed Certification or Seed Law Enforcement Agencies to judge that the seed lot conforms to the prescribed standards.
- For germination test since only the pure seed component is used for germination testing.

## Definitions

**Pure seed:** The pure seed must refer to the species stated by the applicant, or found to predominate in the test, and must include all botanical varieties and cultivars of that species (even if immature, undersized, shriveled, diseased or germinated, providing they can be definitely identified as of that species) unless transformed into visible fungal sclerotia, smut balls or nematode galls, Pure seed shall include:

- a) Intact seed units (commonly found as dispersal units i.e. achenes and similar fruits, schizocarp, florets et.) as defined for each genus or species;
- b) Pieces of seed units larger than one half their original size. From the above main principles certain exceptions are made for particular genera or species as given below:
  1. Seed units of families *Leguminaceae*, *Cruciferae*, *Cupressaceae*, *Pinaceae* and *Taxodiaceae* with the seed coat entirely removed shall be regarded as inert matter, Separated cotyledons of *Leguminaceae* are regarded as inert matter, irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.
  2. In certain genera of family *Gramineae* following exceptions are followed:
    - a. A minimum size of caryopsis is required i.e. in *Lolium*, *Festuca* and *Elytrigia 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 carropsis in spikelets and florets is not always obligatory.
    - c. The separation of pure seed and inert matter is done by uniform blowing procedure. This method is obligatory for *Poa partensis* and *Dactylis glomerata*.
    - d. Multiple seed unit (MSU) are left intact in the pure seed fraction e.g. *Dactylis* and *Festuca*.

- e. Attached sterile florets are not removed, and included in the pure seed fraction e.g. *Arrhenatherium*, *Avena*, *Chloris*, *Dactylis*, *Festuca*, *Holcus*, *Poa*, *Sorghum* and *Triticum spelta*.
- f. For certain genera appendages are left on the seed but reported if found to the extent of 1% or more, the percentage of such material must be shown on Analysis Certificate (example-paddy).

**Other Crop Seeds:** Other Crop Seeds shall include seed units of any plant species other than that of pure seed grown as crops. Multiple structures, capsules, pods are opened and the seeds are taken out and the non seed material is placed in the inert matter.

**Weed Seeds:** Seeds recognized as weeds by laws, official regulations or by general usage shall be considered as weed seeds.

**Inert matter:** Inert matter shall include seed species and all other matter and structures not defined as pure seed excluding other crop and weed seeds.

**General Principles:** As per ISTA Rules, the working sample is separated into three components i.e. pure seeds, other seed, and inert matter. However under IMSCS other seed fraction is further defined as weed seeds, other distinguishable variety (ODV) & other spp. The percentage of each part is determined by weight except weeds seeds & ODVs, which are reported as numbers. All species of seed and each kind of inert matter present shall be identified as far as possible and if required for reporting, its percentage by weight shall be determined.

**Equipments:** Aids such as transmitted light, sieves and blowers may be used in separating the component parts of the working sample. The blower is to be used, for the uniform blowing method, for species of family *Gramineae*. Other equipments required are:

- Dividers
- Analytical balance with at least two decimal weighing provision.
- Diaphnoscope with reflected light to separate inert matter such as empty florets of grasses
- Sieves to remove fine dust particles.
- Sample pans, dishes, forceps, spatula and hand lens
- Seed herbarium of crop and weed seed

**Procedure:** Obtaining working sample: Since the size of the working sample is small is small as compared to the size of the seed lot, which it represents, it is important that the working sample should be obtained in accordance with the prescribed procedures. The

working sample shall be either a weight estimated to contain at least 2,500 seed units or not less than the weight prescribed for a sp. 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 are followed:

- Check the cleanliness of the divider and the container.
- Pour the entire contents of the submitted sample into the hopper of the divider.
- Allow the content 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 opening the gate-valve, situated at the base of the hopper.
- Combine the contents of both receiving pans and again pass it through the divider.
- Repeat this process twice in order to homogenize the submitted sample.
- Divide the submitted sample.
- Set aside the contents of one container.
- Divide the contents of the other container subsequently till the weight of working sample is obtained.

**Separation:**

- Clean the work board and purity dishes before starting the separation
- Examine the working sample with the use of particular aid such as blower or sieves for making separation, if required.
- After preliminary separation with the help of sieves or blower, place and spread the retained portion (A) on the purity work board.
- With the help of spatula or forceps, spread working sample thinly and examine each particle individually. The criteria used being the external appearance (shape, size, colour, gloss, surface texture) in naked eyes in transmitted light.
- 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.
- Seed enclosed in fruits other than those indicated in pure seed should be separated and the detached empty fruit/appendages classed as inert matter.
- Collect the pure seed in the sample pan.
- Put the lighter portion (B) of the work board and examine under magnification for further separating into the requisite classes (other crop seed, weed seed and inert matter).
- After separation, identify the other crop seed, weed seed and record their names on the analysis card. The kind of inert matter present in the sample should also be identified and recorded as far as possible.



- Weigh each component, pure seed, other crop seed and inert matter in grams to the number of decimal places shown below:
- Weed seed may be reported by numbers.
- Other distinguishable varieties (ODV) of the same species may also be recorded, particularly in such crops as rice, soybean, mustard, pulses etc., where maximum limits are prescribed standards. ODV are also reported by numbers.
- Calculate the percentage by weight of each component to one decimal place only, based on the sum of the weight of all the four components. If any component is less than 0.05%, record as 'Trace' or 'TR'. Component of 0.05% to 0.1% are reported as 0.1%.

**Reporting results:** The results of purity test are to be given to one decimal place only and the percentage of all component must total to 100. If the result for a component is nil, this must be shown as 0.0% in the appropriate space of the report form. The report should also include the kind of inert matter and the Latin names of the crop seed and weed seed found in the sample.

| Sl. No. | Wt. of Working Sample (g) | No. of decimal place required | Example |
|---------|---------------------------|-------------------------------|---------|
| 1       | <1.00                     | 4                             | 0.9025  |
| 2       | 1 to 9.990                | 3                             | 9.025   |
| 3       | 10 to 99.99               | 2                             | 90.25   |
| 4       | 100 to 999.9              | 1                             | 902.5   |
| 5       | ≥1000                     | 0                             | 1025    |

**Definition of Pure Seed:** The following structures are counted as pure seeds in different species.

#### Gramineae:

##### Oryza (Paddy)

- Spikelet with glumes, lemma and palea enclosing a caryopsis including the awn, irrespective of its size.
- Floret, with or without lemmas, with lemma and palea enclosing a caryopsis, including the awn irrespective of its size.
- Caryopsis.
- Piece of caryopsis larger than one-half the original size.

Note: Seeds with awns longer than length of floret are reported according ISTA Rule 3.7.

### **Hordeum (Barley)**

- Floret, with lemma and palea enclosing a caryopsis, with or without awn or with or without rachis segment irrespective of their length.
- Piece of floret containing a caryopsis larger than one –half the original size.
- Caryopsis
- Piece of caryopsis larger than one-half of the original size.
- N.B. Florets with awn or rachis segment longer than length of floret are reported according to ISTA Rule 3.7

### **Avena (Oat)**

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

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

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

### **Panicum (Sawa)**

- Spikelet, with glumes, lemma and palea enclosing a caryopsis plus attached sterile lemma.
- Floret with lemma and palea enclosing a caryopsis.
- Caryopsis
- Piece of caryopsis larger than one-half of the original size.
- No need to check the presence of caryopsis

### **Pennisetum (Pearl Millet / Bajra)**

- Panicle of 1-5 spikelets (Spikelets with glumes, lemma and palea enclosing a caryopsis plus attached sterile lemma) with involucre of bristles.
- Floret with lemma and palea enclosing caryopsis.
- Caryopsis.
- Piece of caryopsis larger than one –half of the original size.

### **Sorghum (Jowar)**

- Spikelet, with glumes, lemma and palea enclosing a caryopsis with or without hyaline palea or lemma, rachis segment, pedicel(s), awn(s), attached sterile or fertile floret(s).
- Floret, with lemma and palea, with or without awn.

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

**Leguminosae** (Gram, Pea, Mung, Urd, Bean, Cluster bean, soybean, Lupins, crotolaria (Sunhemp), Medicago, ARchis, Trifolium). **Cruciferae** (Radish, Mustard, Cabbage and cauliflower).

- Piece of seed larger than one-half the original size with testa.
- Intact seed with both cotyledons and testa.

**Solanaceae** (Chillies, Brinjal, Tomato, Tobacco):

**Linaceae** (Linum); **Liliaceae** (Onion, Garlic); **Amaranthaceae** (Amaranththus);

**Cucurbitaceae**: Watermelon, long melon, Musk melon, Cucumber, Pumpkin, Squash, Bottle guard etc.

**Pedaliaceae** (Sesamum)

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

**Umbelliferae**: (Carum, Coriander, Cumin, Carrot, Fennel)

- Schizocarp, which is a dry fruit and separates into two or a more units (mericarps) at maturity.
- Piece of mericarp larger than one-half the original size, unless it is obvious that no seed is present.
- Seed with the pericarp partially or entirely removed.
- Piece of seed larger than one-half the original size, with the pericarp partially or entirely removed.

Note: Fruits with pieces of pedicel longer than the length of schizocarp / mericarp are reported according ISTA Rule 3.7.

**Malvaceae (Cotton)**

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

**Compositeae** (Sunflower, Lettuce, Chicory)

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

- Piece of achene larger than one-half the original size, unless it is obvious that no seed is present.
- Seed with the pericarp/testa partially or entirely removed.
- Piece of seed larger than one-half the original size, with the pericarp/testa partially or entirely removed.

### **Euphorbiaceae (Ricinus)**

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

### **Chenopodiaceae: (Spinach, Beet, Sugar beet, Red beet)**

- Cluster, or piece of cluster, with or without stalk unless it is obvious that no seed is present.
- Seed, with pericarp/testa partially or entirely removed.
- Piece of seed larger than one-half the original size with the pericarp/testa partially or entirely removed.
- Seeds with pieces of stalk protruding more than the width of cluster are reported according to ISTA 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 1% or more, the percentage of such material must be shown on the analysis certificate. In certain genera seeds/fruits may have various appendages (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 Genuineness of Varieties

Seed is the basic input in agricultural industry and plays a crucial role in boosting up the agricultural production as well as economy of the country. In present day agriculture, the investments incurred on other agricultural inputs like fertilizers, irrigation, insecticides and weedicides will pay the expected dividends only if the seeds used are of high quality. Thus the progress in agricultural production of the country depends on the timely availability of required quantities of 'Quality Seed'. The seed, which is having maximum possible physical purity, germinability, seed health and genetic purity, is termed as 'Quality Seed'. Among these attributes, the first three decides the crop stand in the field and the genetic purity governs the maximum yield potential that could be realized from using a particular cultivar.

The term 'Genetic purity/ Cultivar purity' generally infers that plant population of a particular variety is homogenous and genetically identical i.e. true to type with respect to the cultivar it is claimed for. But during seed multiplication, the cultivar purity gets deteriorated because of mechanical admixtures, out crossing, residual segregation and mutation, which at times are unavoidable. So in order to realize the full potential of the cultivar and to retain the farmer's faith in high yielding technology, the seed of improved cultivars should be ensured for their cultivar purity before selling it to farmers.

### Objective of genetic purity testing

The objective of cultivar purity testing is to determine the extent to which the submitted seed sample conforms to the species (using methods not permissible in a purity test) or cultivar claimed for it.

### Field of application

The determination is valid only if the sender of the sample states the species or cultivar and if an authentic standard sample of the species or cultivar is available for comparison. The characters compared may be morphological, physiological, cytological or chemical.

### Means to assess cultivar purity

Cultivar purity testing is mainly based on the detectable differences present between the cultivars. The differences between the cultivars are attributed to genetic differences housed in DNA that in turn is transcribed into a RNA sequences from which a series of amino acids forming polypeptide chain or proteins are translated. Proteins serve as structural components in plant cells and also as enzymes catalyzing both primary and secondary catabolic reaction, the products of which are various organic compounds

including carbohydrates, lipids, phenols etc. These chemical components function in energy storage and cell structure, the latter collectively composing anatomical and subsequent morphological features. Thus the purity of cultivars could be assessed based on the differences in visible morphological characters or differences in chemical nature of respective cultivar. Most of the cultivar purity tests can, therefore, be divided into either morphological or chemical tests, which are as follows:

- Examination of seeds:
  - Examination of seedlings:
    1. Coleoptiles pigmentation
    2. Seedling colour test  
in *Beta spp.*
    3. Colour of cotyledons  
*Brassica spp.* in
    4. Fluorescence test in  
and *Festuca spp*
- } Tests Recommended by ISTA  
*Lolium spp*
- Cytological test (Ploidy test)
  - Field plot test
  - Growth chamber tests
  - Chemical tests:
    1. Phenol test in wheat
    2. Lugol's test for lupin
    3. Electrophoretic techniques in  
wheat, oat, barley, peas, maize,  
sunflower and *Lolium*
    4. Copper sulfate-Ammonia test for  
sweet clover
    5. HCl test for oat
    6. Peroxidase test for soybean
    7. KOH test for rice and sorghum
    8. NaOH test for wheat
- } Tests Recommended by ISTA
- } Tests Recommended by AOSA

### General principles

The determination is carried out, depending on the species or cultivar in question, on

- (a) Seeds,
- (b) Seedlings, or more mature plants grown in a laboratory, a glasshouse, a growth chamber or field plots.

Normally, seeds are compared with seeds from the authentic sample and seedlings and plants are compared with seedlings and plants at the same stage of development grown from the authentic sample contemporaneously, near-by and in identical environmental conditions. If more than one submitted sample is to be verified for the same cultivar/species, it is sufficient to include at least one working sample from authentic sample as a control for every 15 working samples from the submitted samples. Exceptionally, depending on the certainty of the determination (e.g. ploidy), comparison with the authentic control sample is not obligatory.

In the case of species or cultivars that are sufficiently uniform as to one or more diagnostic characters (e.g. in self-pollinated species), a count is made of the number of seeds, seedlings or plants that are not in conformity with the authentic standard sample. If the species or cultivar is not sufficiently uniform (e.g. in cross-pollinated species) a count is made of any obvious off-types and a general judgment is expressed as to the conformity of the sample under test.

### Submitted samples and working samples

#### *Submitted samples*

The minimum weights of submitted samples shall be as follows:

|  | Field plot only (g) | Laboratory and Field plot (g) |          |
|--|---------------------|-------------------------------|----------|
| Pisum, Phaseolus, Vicia, Lupinus, Zea, Glycine, and species of other genera with of similar size | 1000                | 2000                          | seeds    |
| Hordeum, Avena, Triticum" Secale, and species of other genera with seeds of similar size         | 500                 | 1000                          | size     |
| Beta and species of other genera with similar size   | 250                 | 500                           | seeds of |
| All other genera   | 100                 | 250                           |          |

### Examination of seeds

#### *Working sample:*

Not less than 400 seeds, taken at random from a sub-sample drawn in accordance with ISTA seed testing rules. The seeds shall be tested in replicates of not more than 100 seeds. When electrophoresis methods are employed, it is permissible to use smaller

working samples than this. The size of the working sample and the amount of replication needed will depend on the method used and the degree of precision required.

*Determinations:*

- (a) For morphological characters, the seeds shall be examined with the aid of suitable magnifying apparatus when necessary.
- (b) For colour characters, the seeds may be examined under full daylight or light of limited spectrum, e.g. ultra-violet.
- (c) For chemical characteristics, the seeds shall be treated with the appropriate reagent and the reaction of each seed noted.

### **Examination of seedlings**

*Working sample:*

Not less than 400 seeds (or for ploidy initially, 100 with a further 100 when the initial determination is inconclusive), taken at random from a sub-sample drawn in accordance ISTA seed testing rules.

*Determination:*

The seeds shall be germinated in replicates of not more than 100, on an appropriate medium. When the seedlings have reached a suitable stage of development, they are examined in whole or in part, with or without further treatment. For a determination of ploidy, root tip or other tissue is excised and processed for microscopic examination.

### **Examination of plants in glasshouse or growth chamber**

*Working sample:*

Sufficient seeds to produce not less than 100 plants, but this number may be reduced in the case of climbing or creeping species. The seeds shall be taken at random from a sub sample drawn in accordance with ISTA seed testing rules.

*Determination:*

The seeds shall be sown in suitable containers and maintained in the environmental conditions necessary for the development of diagnostic characters. When the plants have reached a suitable stage of development, the critical characters shall be observed on each plant and noted.

### **Examination of plants in field plots**

The submitted sample shall be sown (in whole or in part) as soon as practicable after receipt. Each sample shall be sown in at least two replicate plots. As insurance against failure the replicates should be situated in different fields or different parts of the same field. The plots may be of any convenient size that will provide enough plants for the



determination to be of the accuracy required. If the seed is sown in situ, it shall be sown in rows, mechanically if possible. Spacing between rows and between plants shall be sufficient to allow development of the characters to be examined. Both transplanting and thinning are possible sources of error and the sowing rate shall be adjusted to produce approximately the same number of plants in the test and control plots. When absolutely necessary, thinning or transplanting of seedlings from elsewhere in the plot is permissible. Observations shall be made during the whole growing period and deviations from the control sample recorded. Plants recognisable as belonging to another cultivar or species or as aberrants (e.g. fatuoid oats, speltoid wheats) shall be counted and recorded.

When practicable, either an actual count or an estimate of the number of plants in the plot shall be made, preferably at the time the plants are examined.

### **Calculation and expression of results**

When not more than 2000 seeds, seedlings or plants are examined, the number found to be not genuine is computed as a percentage without decimals. If more than 2000 are examined, the number is computed as a percentage to one decimal place.

*Seeds and seedlings:* In determinations of seeds and seedlings, the results are expressed as percentages of the number of normal seedlings examined.

*Field plot examination:* Whenever possible, the number of plants found to be of other cultivars, other species or aberrant shall be calculated as a percentage of the number of plants examined.

In the case of herbage plants and similar species when grown in rows without wide spacing, it is difficult to estimate the total number of plants examined per plot and- the result may be expressed as the number of divergent plants produced by the weight of seed sown. When characters are measured, the mean and other statistics may be calculated.

Cultivars of cross-fertilizing species such as rye, root crops, herbage plants etc. often show variability of plant characteristics to such a degree that it is difficult to define accurately all off-types; in such cases, any calculations of percentage impurities shall be supplemented by appropriate comments about the conformity of the test sample to the authentic standard sample.

### **Reporting results**

The results shall be reported under 'Other Determinations' on an ISTA International Seed Analysis Certificates as follows:

1. For laboratory, glasshouse or growth chamber tests, the number of seeds, seedlings or plants examined shall be stated.

The result of an examination of seeds or seedlings shall be reported as the percentage of non-conforming seeds or seedlings. If none is found, the result shall be reported as follows:

"The laboratory (or glasshouse) examination for conformity with the authentic standard sample revealed nothing to indicate that the species and cultivar stated by the sender are incorrect"

2. In the case of a fluorescence test of *Lolium*, the result shall be reported as follows: "Of . . . seeds producing normal seedlings, . . . % reacted positively and. . . % reacted negatively to ultra-violet light"

3. Results of electrophoretic analysis shall be reported as:  
"Of . . . seeds examined. . . seeds were not of the variety stated."

4. The result of a field plot examination shall, whenever possible, be reported as a percentage of each other species, other cultivars or aberrants found. If a sample is found to be of a cultivar other than that stated by the applicant, this result shall be reported. If the proportion of plants of other cultivars present in a sample exceeds 15%, the report shall state additionally that: "The sample consists of a mixture of different cultivars".

When the expression of the result as a percentage is not possible, appropriate comments regarding the conformity of the sample may be reported. If nothing worthy of special comment was found the following statement is required:

The results of a field plot examination of this sample revealed nothing to indicate that the cultivar (or species) name stated by the sender is incorrect

If, exceptionally, an authentic control sample has not been tested for comparison, this shall be stated.

### **Biochemical Tests**

With increasing number of varieties and limited diversity for morphological characters, it is difficult to establish the identity and distinctness of a variety and instead can be categorized into different groups. This is more so when newer varieties are developed using germplasm with limited level of genetic diversity or when convergent selection towards similar morphology is practiced. For such situations, inclusions of additional biochemical markers are often found useful. Application of biochemical tests like phenol colour reaction, peroxidase test and electrophoresis techniques for the said purpose has been discussed below.

### Phenol Colour Reaction Test

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

Figure 1: Seed Coat Phenol Colour Reaction in wheat



### Peroxidase Test

Peroxidase test is based on the activity of peroxidase isozyme present in the seed coat and is widely used for variety characterization, identification and purity testing in soybean. The cultivars are identified based on either low or high seed coat peroxidase activity, which is confirmed by the colour change obtained through oxidation of guaiacol reagent added to the samples in the presence of hydrogen peroxide (Fig. 2). This test was first used for variety identification in soybean varieties, which could be grouped into two

groups based on the peroxidase activity. Later this test was expanded to other crops like pea, black gram, cotton, chickpea and pearl millet.

Figure 2: Peroxidase Test Results in Soybean



### Electrophoresis Techniques

The successful exploitation of electrophoresis for plant variety characterization, identification and variety purity testing relies on the fact that the proteins/isoenzymes are the structural products of genes. Proteins/isoenzymes can thus be regarded as markers for the structural genes that encode them. Besides, the analysis of electrophoretic profiles of protein /isozymes is not only rapid, less labor intensive than traditional methods but also more reliable since their expression is not altered by environmental factors. Thus the electrophoresis technique could be used as an effective tool to identify varieties and for testing genuineness of varieties, since each variety differs from other varieties in one or more protein and isoenzyme constituents. Hence, this technique is being widely used for variety identification and purity testing in large number of agricultural crops.

In this technique, proteins/Isoenzymes are separated into distinct bands in a support medium of polyacrylamide or starch gel under the influence of electric current applied across the medium. The separation is due to the differences in the size/charge/both of the protein/isoenzyme involved. The difference between the varieties is established based on presence or absence of a particular protein/isozyme band at a particular position in a support medium, which is marked by Relative mobility (R<sub>m</sub>) value of that particular band.

### Protein Markers

The composition of the seed proteins is highly constant and is unlikely to be affected by environmental conditions or seasonal fluctuations. Since most of the released varieties have almost similar morphological characters, the feasibility of this marker is greater as compared to morphological marker. The most commonly observed differences among varieties are total number of protein bands and relative number of bands in a given region of the separating media (generally acrylamide or starch) making it an effective tool for

testing the genuineness of cultivars. Applicability of this marker for variety characterization, identification and purity testing has been well demonstrated in different crops.

### **Isoenzyme Markers**

Analysis of seed proteins may, at times, yield a number of bands and interpretation of results may be difficult. It might then be more advantageous to look for polymorphism in isozymes, which are coded by fewer loci. Isozymes are the multiple forms of an enzyme having similar or identical catalytic activities. The use of isozymes as a co-dominant marker for variety characterization, identification and purity testing has been adequately reviewed and established that it has high utility for variety identification and purity testing, which is well established in many crops.

### **Application of electrophoretic technique for testing variety purity and identity**

ISTA has accepted electrophoretic technique for testing the genuineness of varieties in crops like wheat, oat, barley, maize, peas and sunflower (ISTA 2004). While testing the purity of a variety the electrophoretic profile developed from individual seed/seedling is compared with the profile developed from the authentic sample of that particular variety. Even though electrophoretic technique proved to be quickest method to ensure the purity of seed lot, it is not widely applied, the reasons being, the cost involved, expertise required and non-availability of standard methods for analysis of protein/isozymes from single seed in many crops. Attempts are being made to make it cost effective by reducing the size of sample to be analyzed through application of sequential sampling methods.

### **Molecular Markers**

The ability of protein/isozymes to discriminate between genotypes is generally limited due to a small number of loci and insufficient polymorphism among closely related genotypes. Further the morphological markers and isozymes have the disadvantage of limited expression, which is restricted to specific developmental stages. In recent past, DNA based markers have been applied for this purpose, which precisely assay variation in the nucleotide sequences covering the greater proportion of plant genome and provides wider genomic markers. Moreover such differences remain unaffected across different growth stages, seasons, locations and agronomic practices. As the molecular markers are unlimited in number, a thorough sampling of genome is possible. Therefore variety characterization, identification and purity analysis become more reproducible and objective. The advent of several methods for DNA analysis has widened the possibilities of applying such technologies for the purpose. Some of these are listed below:

- Restriction Fragment Length Polymorphism (RFLP) (Probe based marker)
  - Random Amplified Polymorphic DNA (RAPD)
  - Amplified Fragment Length Polymorphism (AFLP)
  - Simple Sequence Repeats (SSR)
  - Cleaved Amplified Polymorphic Sequence (CAPS)
- } Polymerase Chain Reaction (PCR) based markers

### **Principle:**

To assess the varietal purity of the respective seed lots, the DNA is extracted from the individual seeds/seedlings/plant tissues taken at random from the submitted sample and the DNA profile is generated using specific markers. Thus developed DNA profile is compared with the DNA profile developed from the authentic sample (the variety for which the seed lot is claimed for). Based on the presence/absence of the particular bands in the profile of the sample, the number of offtypes/selfed seeds in case of hybrids will be counted and expressed in percentage of total seeds/seedlings/plants analyzed.

### **Restriction Fragment Length Polymorphism (RFPL):**

Detection of RFLPs was the first DNA-based method that revealed numerous polymorphisms that were inherited in a simple mendelian fashion. Its applicability in crop improvement, variety characterization, identification, protection and its ability to generate highly specific fingerprints has been well demonstrated. RFLPs provide a higher level of discriminatory power than the biochemical markers in many crops like soybean and it was found to be very useful marker in discriminating very closely related inbred lines in maize. Despite these advantages, RFLP analysis is slow and requires relatively large amounts of plant material, intensive labour support and much laboratory space, making it very expensive. Further, in comparison to isozymes, RFLP requires more time and cost per sample analysis. Besides, this marker has been found to be not suitable in crops like tomato and wheat. The use of radioactivity material ( $^{32}\text{P}$ ) for labeling the probes limits the frequent use of this technique. For these reasons, methods based on RFLPs are not applied for variety purity purposes.

### **Polymerase chain reaction (PCR)-based methods:**

PCR-based methods offer new opportunities for genetic purity analysis since small amounts of DNA are required and DNA profiles can be obtained more quickly than with RFLPs (in days rather than weeks). PCR-based methods such as RAPDs, amplified fragment length polymorphisms or microsatellites (also called simple sequence repeats (SSRs)) are more cost-effective than RFLPs and faster but are subject to the relatively high expense of thermostable polymerases and the time, personnel and space needed to run and score numerous gels.

### **Random amplified polymorphic DNA (RAPD):**

The main advantage of RAPD marker is that, it does not require the prior knowledge of the target DNA sequences or the prior development of the markers. Hence it is the ideal marker to initiate the efforts in finding the suitable alternative for variety characterization, identification and purity testing. RAPD has been applied for establishing distinctness and identity of varieties in many crop species like barley, oats, onion, cabbage, wheat, etc. However, its reliability for this application is doubted because this marker is reported to provide irreproducible and sometimes unexpected. Moreover, the low degree of complementarity between primer and target DNA sequence leads to differing results even in the same laboratory and hence makes standardization of the test extremely difficult. The relatively low primer annealing stringency used in RAPD analysis apparently also results in lack of amplification of some parental bands in the F<sub>1</sub> hybrid. Overall, lack of reproducible results greatly compromises the accuracy and the practicality of using RAPDs for purity analysis. The ability to obtain robust data from RAPDs can be increased provided great care is taken to monitor DNA amplification cycles, to standardize steps of PCR and DNA concentration. Satisfactory repeatability can usually be attained for samples amplified within at least the same laboratory provided immense care is taken and check samples are included to evaluate thoroughly variations in amplification that do not have a genetic basis. However, usually it will be necessary to include standard genotypes of assured high purity along with each set of individuals of the variety being assayed to facilitate correct data interpretation. Since RAPD data are easy to generate, one can come across large number of publications using this methodology. However the quality and interpretation of such data should be critically reviewed and this method should be used with great care and only when no other method is practically feasible.

### **Amplified fragment length polymorphisms (AFLPs):**

Like RAPD, AFLP does not require the prior knowledge of the target DNA sequences or the prior development of the markers. In comparison to RAPD markers, AFLP markers have more discrimination power. This is due to the fact that number of bands generated using single AFLP marker is nearly ten times more. AFLP technique circumvents most of RAPD drawbacks by using high stringency PCR primer annealing conditions to known DNA sequences that are ligated onto restriction fragments. AFLPs, however, continue to be a problem in that inter-laboratory variability remains an issue for variety characterization, identification and purity testing. To conclude, neither RAPDs nor AFLPs produce data that can be unambiguously and readily scored as co-dominant alleles at mapped loci. This limitation poses a practical problem for genetic purity assays since outcrosses can then remain undetected.

### **Simple sequence repeats (SSRs) or microsatellites:**

STMS (Sequence Tagged Microsatellite Sites) markers have the advantages of simplicity, rapidness, reproducibility and cost effectiveness compared to RFLP, RAPD and

AFLP markers. STMS markers are having wide potential to be used for genetic purity testing due to their hyper variability, co-dominance, dispersion throughout genomes and suitability for automation. However, in contrast to RAPDs and AFLPs, SSR technology is initially expensive to implement and the method must be independently initiated for most individual species. DNA sequences from the target species must be obtained and screened for di-, tri-, or tetra-nucleotide repeat motifs occurring in tandem arrays. The regions flanking each tandem array are then sequenced, and primers are designed for amplification of the intervening repeat region. Primer design and marker screening for polymorphisms among varieties of the species of interest are essentially required, regardless of whether the starting point of the experiment is enriched DNA repeat libraries or sequence databases. This is because (i) some SSRs will be more polymorphic than others across the varieties of interest; (ii) map information must be obtained and subsequently considered; and (iii) assessment of the robustness of amplification reactions must be carried out empirically. Although the start-up costs are high, potential savings through use of automation and reduction in reaction volumes exist for this marker.



## Seed production in maize (OPV/Hybrids): Field and Seed standards

### Introduction

Maize (*Zea mays* L.) is the multi utility crop with wider adaptability and highest genetic yield potential among the cereals. It is an important cereal crop in world after wheat and rice. Maize is unique among the cereals on account of various features which make it as one of the top three cereals and its amenability to diverse uses is unparalleled. Ranging from pharmaceuticals to many other industrial uses like biofuel, besides food, feed and fodder purpose, diverse corns find their place. There are many types of maize based on the grain composition such as dent, flint, pop, pod, waxy and floury maize. Maize occupies an important place in world agriculture. It is grown in more than 150 countries. The major producing countries are USA , China, Brazil , Argentina, Mexico , India . At global level, India ranks 4<sup>th</sup> in area and 6<sup>th</sup> in production of maize. In India as per the latest report, maize area, production and productivity is 8.71 mha, 22.23mt and 2.55 t/ha, respectively during 2012-13 (DAC, 2012). Maize productivity is relatively higher in the states like Karnataka, Andhra Pradesh, Bihar, Punjab and Himachal Pradesh. The productivity of Andhra Pradesh is highest in India. Utility pattern of maize in India are as source of human food 25%, as animal feed 12%, in poultry feed 49%, in starch industry 12%, brewery 1% and as seed 1%.

Maize is widely cultivated throughout the world, and a greater weight of maize is produced each year than any other grain. In Asia maize is widely cultivated in many countries and among the ASEAN countries leading producer of maize is Indonesia followed by Philippines, Thailand and Myanmar (Table-1). Production can be significantly could increased in rest of the ASEAN countries through mutual benefit sharing programmes. For this, sharing of expertise in the field of seed production in maize have huge potential.

**Table-1 Area, production and productivity of world, Asia, India and ASEAN countries during 2012. (source FAOSTAT, 2012)**

| Country                    | Yield (Kg/Ha) | Production (M. tons) | Area (in Ha) |
|----------------------------|---------------|----------------------|--------------|
| <b>I. World</b>            | 4944          | 875.10               | 176.99       |
| <b>II. Asia</b>            | 5007          | 287.92               | 57.49        |
| <b>III. India</b>          | 2507          | 21.06                | 8.40         |
| <b>IV. ASEAN Countries</b> |               |                      |              |
| 1. Indonesia               | 4893          | 19.38                | 3.95         |
| 2. Malaysia                | 5200          | 0.05                 | 0.01         |

|                |      |      |      |
|----------------|------|------|------|
| 3. Philippines | 2856 | 7.41 | 2.59 |
| 4. Singapore   | --   | --   | --   |
| 5. Thailand    | 4457 | 4.81 | 1.08 |
| 6. Brunei      | --   | --   | --   |
| 7. Myanmar     | 3947 | 1.50 | 0.38 |
| 8. Cambodia    | 2394 | 0.79 | 0.33 |
| 9. Laos        | 5719 | 1.13 | 0.20 |
| 10. Vietnam    | 4295 | 4.80 | 1.12 |

### Maize breeding research in India

The focused research in SCH helped in addressing several issues of biotic and abiotic stresses *viz.*, lowering water table, rising temperature, etc. The success story of SCH in US Corn belt is well known. Its impact has been realized in China, Brazil, Canada and many other countries too. Even in USA with cultivation of Open Pollinated Varieties the productivity remained less than 2 tons/ha. And further, the results were not encouraging with the coverage of 100% area under double cross hybrids and the productivity was only 3.5 tons/ha over a period of 25 years (1936-1930). But with the adoption of SCH technology in 1960s USA productivity increased 3.5 tons/ha (1960) to 9.68 tons/ha (2008). The annual increase in productivity with 100% coverage under double cross hybrid was only 60kg/annum in 25 years and with single cross hybrid cultivation the productivity per annum is more than double in a period of 50 years. Parallel to USA in India the productivity remained less than 1 ton/ha for many decades continued. After shifting to SCH technology (2006-2008), in India has witnessed 30% increase in production and 27% increase in productivity within two years with the coverage of 20% area under SCH. There is also 15% annual increase in production and more than 12% increase in productivity. India became net importer to potential exporter. This is the visible impact of single cross hybrid technology. So maize breeding strategies in different period of time (Table-2) has been adopted in India as follows

**Table-2 maize breeding strategies in different period**

| Period  | Maize Breeding Strategies |
|---------|---------------------------|
| 1950-60 | Land races                |
| 1960-67 | Double cross (DC)         |

|              |   |
|--------------|---|
| 1967-71      | Composite   |
| 1971-89      | Composite and Double top cross (DTC) / DC                           |
| 1989-2000    | Single Cross Hybrid (SCH)/DC / Three Way cross (TWC) and composites |
| 2000-06      | SCH/TWC/DC and composites   |
| 2006-onwards | Single Cross Hybrids  |

## Reproductive biology:

### Inflorescence

Maize is a monoecious plant i.e. male and female inflorescences are located at separate places on the same plant which cross pollination a general rule. However, five per cent self – pollination is also recorded. The male flowers are borne in cluster (called tassel) on the top of the stem as a terminal panicle. The branches of the tassel are spirally arranged around the axis. The female flowers are borne inside the young cobs, wrapped under bracts, which arises from one of the nodes on the stem usually located about midway on the stalk.

### Male Flower

The spikelets are usually arranged in pairs one sessile and the other pedicellate (stalked). Each spikelet is enclosed by two glumes. There are two functional florets per spikelet. Each floret is enclosed between the lemma and plea and contains three stamens with linear and pendulous anthers, two small cup –shaped lodicules and rudimentary pistil (weather wax 1955).

### Female flower

The female spikelets are densely packed in several vertical series on the thick and cylindrical rachis. Each spikelet is enclosed two membranous, broad and empty glumes. Lodicules are absent or very feebly developed. the spikelet has a lower barren ( extremely reduced) and an upper fertile floret. Each floret enclosed between lemma and plea (Dutta 1971). The style is very long silky filament and in the cluster is known as silk.

Silk become receptive as soon as they emerge from the ear husk. Generally, silk grow upto 10-15 cm in length and can retain viability upto 7-10 days in want of effective pollination (Walden and Everett 1961). Best seed sets occurred with pollinations three to five days after first silk emergence, but pollination after eight days still gave 66 percent seed set compared with optimum ( Hallaver and Sears 1966).

### Anthesis

At anthesis ,just prior to pollen shedding, the lodicules swell to several times of their normal size and push the plea and lemma apart, facilitating the anthers exertion through filament elongation. Anthers open at the tip, forming pores through which the pollen mass

is discharged in huge numbers in wind. Moisture stress and high temperature of about 35-40 degree centigrade may also cause tassel firing. It is estimated that a tassel produces 25000 pollen grains for each female gamete in a normal environment (Kiesselbach 1994). After release and dispersal from anthers, pollen grains retain viability for few minutes only.

### **Synchronization of flowering**

Split date plantings of seed parents refer to the planting of the female and male parents on different dates. This practice is employed to optimize the synchronization of pollen shed and silking of the two seed parents of different maturity "nick " or reach the flowering stage concurrently ( Wych, 1988). Male parents are often planted on two dates to extend the pollen –shedding period by the inbred male. Plantings are timed so that peak pollen shed coincides with the maximum exposure of silks by the female parent. Other methods utilization to alter flowering dates to bring parents of differing maturities together for timely nick include clipping of flaming to delay crop development, variable planting depths, and variable fertilizer rates. The methods are not used widely because they can reduce seed yields.

**In maize crop following kinds of varieties/hybrids are being commercially growing:**

**E. Open-pollinated varieties**

**F. Synthetic varieties**

**G. Composite**

**H. Hybrid**

#### **A. Open-pollinated varieties**

OPVs are made up of genotypes that are selected based on phenotypic appearance and bulked without testing progeny performance previously or performance in hybrid combinations. Subsequent maintenance of the variety should be by open pollination, in isolated field.

#### **B. Synthetic varieties**

A variety synthesized by crossing inter-se a number of genotypes selected for good combining ability in all possible combinations, with subsequent maintenance of the variety by open pollination is known as synthetic variety (Allard, 1960). The genotypes that are hybridized to produce a synthetic variety can be inbred lines, clones, mass selected population or various other materials. Synthetics derived from early generation inbred lines have given encouraging performance in maize crop.

Synthetic varieties in maize widely used due to the following advantages (Allard, 1960):

The presence of more variability in synthetic varieties when compare to double cross hybrids might allow more adaptable to changeable growing conditions, pest and disease infestations. Cost of synthetic variety is lower than hybrid seed cost due to which small and marginal farmers can adopt the synthetic varieties than hybrids.

## C. Composite varieties

The term composite variety refers to a germplasm composite which is commonly used to designate a broad group of materials mixed together in many different ways, and include breeding materials put together on the basis of desirable characters, such as yield potential, maturity, disease resistance etc., followed by random mating. It was in India that a population improvement methodology was outlined by Dhawan (1963) for developing commercial varieties named as composite.

## D. Hybrids

Hybrid seed of maize may generally be produced from following different cross-combinations:

5. Single cross hybrid ( $A \times B$ )
6. Three-way cross hybrid  $(A \times B) \times C$
7. Double cross hybrid  $(A \times B) (C \times D)$
8. Double-top cross hybrid  $(A \times B) \times \text{Composite variety}$

### 1. Single Cross Hybrid

It is a product of the cross between two potential inbred lines (A B). It is highly uniform and heterotic requires three isolations for seed production. However, seed cost is more since the seed yield is less.

### 2. Three Way Cross Hybrid

The hybrid is produced by crossing the F<sub>1</sub> of the cross A, B with another potential inbred line C. This type of hybrid seed production requires five isolations. Generally, three-way cross hybrid is produced where three inbred lines, which combine well, are available but fourth suitable inbred is lacking, otherwise, double cross is more economical.

### 3. Double Cross Hybrid

Is a product of four potential inbred lines. Product of (A B) is crossed between the F<sub>1</sub> of (C D). IT requires seven isolations for seed production and seed cost is less and it is less uniform.

### 4. Double top cross

The first generation resulting from the controlled crossing of a certified single cross and a certified open-pollinated variety. That is, One single-cross and one open-pollinated variety (OPV) or composite variety, are involved in this system. A hybrid seed from single cross is taken as female parent and OPV as male parent.

Single crosses (between two inbreds) are the best with respect to the level of performance and uniformity and have a great merit in the commercial seed production. The genetic purity of inbred parents can be easily maintained and genetically true to type F<sub>1</sub> seed can be produced year after year. In conventional, hybrid seed production, one of the major problems encountered has been the lack of good vigorous inbred lines. All hybrids other

than single cross hybrid have heterogeneous F1 generation, and heterogeneity increases with the number of parent involved. The single crosses have greatest attractiveness and phenotypic appeal, uniformity in kernel type and suitability for combine harvesting. However, being uniform, they lack population buffering and possess only individual buffering, whereas three-way and double crosses have both population as well as individual buffering (Allard and Bradshaw 1964)

### Maize hybrid seed production consists of three stages (Table-3)

(Every stage of seed production is carried out in isolation)

**Table:3 three stages of hybrid seed production in maize**

| Stage of seed productions | Particulars   | Remarks  |
|---------------------------|---|--|
| 1. Breeder seed           | Parental lines are increased in limited area  | Parents should have genetic purity and certifying standards.   |
| 2.Foundation seed         | The seed obtained on male and female rows is called foundation seed   | Parents should have genetic purity and Certifying standards.   |
| 3. Certified seed         | Male and Female single crosses are generally sown in 2:4 ratio. The seed obtained on female parent is called certified Seed or Hybrid seed. | Detasseling should be attended in all female plants at proper time. Both single crosses (Male & Female) should posses genetic purity and certifying standards. |

### For a successful Hybrid seed production following pre –requisites are required

- Male and female parental lines.
- Knowledge of flowering behaviour.
- Proven crossing techniques.

When commercial maize for 100000 ha has to be produced 0.2 ha area of breeder seed production and 10 ha of foundation seed production and nearly 667 ha of certified seed production is required. In Maize three different kinds of hybrids can be produced, that is the breeder seed is produced by the original breeder under his purview. While foundation seed production is taken up state Seeds Corporation, etc., and the certified seed production (F1 hybrid seed production) is usually done in the farmer's field (seed growers). Foundation and Certified seed production is done under the supervision of the Certification Agency.

## **Agronomic practices followed during seed production in maize**

### **Climatic Conditions**

Clear environment, with ample sunshine is the ideal place. However, very high or very low temperature during seed production period is harmful as high temperature (>42 C) results in wide gap between anthesis and silking (ASI), Hence poor seed set. While very low temperature causes improper pollen shedding and seed setting. Therefore an optimum temperature of 21 C for germination 32 C for plant growth is suited.

### **Seed Production Site**

Seed production should be taken in well drained, weed and diseases free soil and preferably the fields where preceding crop was not maize to minimize roguing and maintain the genetic purity.

### **Land Preparation**

The land should be level, fertile, well drained and pH of 5.5 to 7.5 is congenial free from weeds and previous crop should not be maize on the same piece of land. Land should be brought to a good tilth with 2-3 ploughings and harrowing.

### **Time of Sowing**

Appropriate time of sowing is very important for better crop establishment. For most part of India, first week of July during kharif and first week of November during rabi are the optimum time of sowing to avoid flowering from heavy rains during kharif and low temperature should not coincide with flowering. Rains during flowering wash the pollen in kharif and low temperature during winter causes mortality and killing another.

### **Method of sowing and layout**

It is desirable to plant the crop on ridges. Sowing should be done on the southern side of the east-west ridges, which helps in good germination. Planting should be done at proper spacing. Optimum row and plant spacing should be kept at 60 and 20 cm, respectively. This spacing will ease the movement in the field for roguing and removal of tassels. Proper spacing also helps in improving the test weight. Identification labels/tags should be put on the male and female lines to distinguish between them.

The male line has to be sown first for that the lines where male lines have to be sown are to be marked with a peg and later the female lines have to be sown in proper row proportions. All along the border 4 rows of male line have to be sown so that it will act as a natural barrier for pollen from other commercial / seed production Maize plots and also supply pollen. Critical points to be taken note-

- Taking note of male and female seed bags
- Removing the tag keeping it safely for source verification along with the bill.
- Sowing the male lines first, at the marked lines.
- Then sowing the female lines
- Following proper cultural practice
- Removing of types at pre – flowering stage based on tassel colour, silk colour etc.
- Synchronization is not usually a problem in maize. However, if present spray 2% urea to late entry. Three methods of sowing are commonly followed under Indian conditions.



### Seed Rate

One should ensure that the seed viable and free from external infections. Information on seed germination should be obtained for deciding the quantity of seed to be used. Seed germination standard should be 99 to 100% and the quantity of seed required for dibbling method is about 15 kg/ha (female: 10 Kg/ha Male: 5kg/ha). The seed rate should be so adjusted as to obtain the desired plant population. The optimum plant population for achieving high yield is around 65 thousand plants/ha.



**Pest and diseases in maize (given in table-4)****Table-4: Important Pest and diseases on Maize and their control measures**

| <b>Pest</b>     | <b>Control Measure by spraying</b>           |
|-----------------|--|
| Stem borer      | Quinolphos 25 EC -2 ml /ltr                  |
| Ear head bugs   | Carbary 14% -dusting                         |
| Army worm       | Nuvacron poison bait                         |
| Thrips & aphids | Dimethoate spray -1.7ml/ltr                  |
| <b>Disease</b>  | <b>Control Measure</b>                       |
| Rust            | Spray Zineb / mancozeb @ 2.5 gm/ltr of water |
| Leaf blight     | Seed treatment with metalaxzyl 3g/kg deed    |
| Downy mildew    | Ridomil MZspray                              |

**Field standards followed during seed production (Seed certification standards) of open- Pollinated Varieties, Synthetics and Composites****1. Isolation**

In seed production, three isolations, five isolations and seven isolations are required for single cross, three way cross and double cross hybrids respectively (Table ). Hybrid crop raised from single cross at farmers' field is more uniform than double cross hybrid. Single cross seed production is a two stage process whereas double cross has three stages. Due to all these factors, double cross seed production needs greater planning and coordination. Once the barrier of low seed yield of inbred lines are overcome, the single crosses are just natural among the conventional hybrids (Vasal et al. 1995).

**1a. Isolation for OPV s, Synthetics and Composites.**

The seed field shall be isolated from the contaminants shown in column 1 of the Table below by the distances specified in column 2 and 3 of the said in table 5:

**Table-5**

| <b>Contaminants</b>   | <b>Minimum distance (meters)</b> |                  |
|---|----------------------------------|------------------|
|   | <b>Foundation</b>                | <b>Certified</b> |
| Field of other varieties  | <b>400</b>                       | <b>200</b>       |
| Field of the same varieties not conforming to varietal purity requirements for certification and teosinte | <b>400</b>                       | <b>200</b>       |

**(Source: Indian seed certification standards, 2013)**

**1b. Isolation for Hybrids is mentioned in table -6****Table-6: Isolation blocks need if all three generations of seed multiplications are being taken at one place**

| Hybrid type                      |       | Number of isolations  |
|----------------------------------|-------|---|
| 1. Single cross (A × B)          | Three | First tow isolation for two inbreds, breeder seed and foundation seed production.<br>Third isolation for certified seed production (A×B).   |
| 2.Three-way cross (A × B) × C    | Five  | Three isolations for three inbreds (breeder and foundation seed production).<br>One isolation for F <sub>1</sub> seed production (A×B) as foundation seed. One isolation for production certified seed (A × B) × C  |
| 3.Double Top cross (A × B) × OPV | Five  | Three isolations for two inbreds and one OPV (breeder and foundation seed production).<br>One separate isolation for producing F <sub>1</sub> i.e., (A×B) crossed foundation seeds. One isolation for production of certified seed [(A × B) × OPV].   |
| 4.Double cross (A × B) (C × D)   | Seven | Four isolation for seed increase of the four inbred lines i.e. A,B,C and D (Breeder and foundation seed).<br>Two isolation for seed production of the tow parental single-cross hybrids (Foundation seed), i.e. (A×B) and (C×D).<br>One isolation for certified double cross hybrid i.e. (A×B) × (C×D). |

A specific Hybrid of maize shall be isolated from the contaminants shown in column 1 of the table below by the distances specified in column 2 of the said Table -7 :

**Table-7:**

| Contaminants   | Minimum distance (meters) |
|--|---------------------------|
| *Field of any maize with same kernel colour and texture  | 200                       |
| Field of any maize with different kernel colour and texture ,and teosinte                                    | 300                       |
| *field of the same hybrid (code designation)not confirming to varietal purity requirements for certification | 200                       |

|   |     |
|---|-----|
| *field of the other hybrids having common male parent and conforming to varietal purity to varietal purity requirements for certification | 5   |
| *field of the other hybrids having common male parent and not confirming to varietal purity requirements for certification                | 200 |

(Source: Indian seed certification standards, 2013)

### Specific requirements of OPVs, synthetics and composites given in table -8

Table:8

| Factor  | Maximum permitted (%) |           |
|---|-----------------------|-----------|
|   | Foundation            | Certified |
| Off -type plants that have shed of shedding pollen at any one inspection during flowering when 5.0% or more of the plants in the seed field have receptive silks. | 1.0                   | 1.0       |

(Source: Indian seed certification standards, 2013)

### Specific requirements of Hybrids given in table-9

Table-9

| Factor  | Maximum permitted (%) |
|---|-----------------------|
| Offtype plants that have shed or are shedding pollen in male parent at any one inspection during flowering when 5.0% of more of the plants in the seed field have receptive silks.    | 0.50                  |
| Tassels of the plants that have shed or shedding pollen in seed parent at any one inspection during flowering when 5.0% or more of the plants in the seed parent have receptive silks | 1.00                  |
| Total of pollen shedding tassels including tassels that have shed pollens for all three inspections conducted during flowering on different dates                                     | 2.0                   |

|   |      |
|---|------|
| Off-types plants in seed parent at final inspection | 0.50 |
|---|------|

(Source: Indian seed certification standards, 2013)

## 2. Roguing

Roguing helps in maintaining the genetic purity of seeds. During the seed production of maize, strict rouging must be exercised. Fields are regularly inspected and off-types and doubtful plant are discarded before pollen is shed. Based on these observations the off type plants should be removed both in male and female lines before they shed the pollen. Normally, off-types and admixture plants are vigorous and easy to identify. Inbred lines, under of nodes, amount of chlorophyll, number of tassel branches, anther and silk colour, ear length, ear diameter, number of kernels per row and rows of ears kernel colour etc.

### Identifying dissimilar plant rouging



### Pulling out dissimilar plant



Generally rouging is done three times in maize. however, rouging is carried out depending upon the necessity. It is necessary to know the distinguishing features of the variety for effective rouging.

- First rouging should be done during vegetative stage, based on the height of the plant, colour of petiole and colour leaf.
- During flowering stage, second rouging is done based on colour of tassel and silk.
- Finally, before harvest, based on colour of seed and cob characteristics, rouging can be done.
- During drying of the cobs, rouging of cob based on seed colour and seed row will maintain the genetic purity.

**Attention:** During rouging at flowering stage, the off types should be removed away from the field immediately. Otherwise it will contaminate the silk and affect the genetic purity.

**Table-10 Characters to be observed at seed production plots**

| Characters | Parameters   |
|------------|--|
| Plant type | Height : tall/Dwarf<br>Stem: Pigmented / non pigmented   |
| Tassel     | Colour of glumes<br>Colour of anthers<br>Type : Compact or open<br>Silk Colour of silk : Green<br>pink /purple |
| Ear        | Type : Flint /semi flint /Dent<br>Colour : orange/yellow/yellow-orange<br>Cob: white pink                      |

### 3. Detasseling an Important Operation

Removing the tassel before it sheds the pollen from female lines in a seed production plot is known detasseling operation and it should be done before anthesis. It should be practiced row-wise. One person should follow to monitor the each row to check that no part of the tassel is left inside. The process of detasseling should continue for 8-10 days. While detasseling, leaf should not be removed which will otherwise reduce the photosynthesis. It has been observed that the removal of 1 to 3 leaves along with tassel reduces 5-15% yield. The removed tassel should not be thrown in the field but fed to the cattle as it is nutritive fodder.



**Ways of detasseling**

It is very important operation for hybrid seed production of maize. Procedure for this operation as follows:

- Tassel which are going to shed the pollen next day have to be identified.
- They have to be removed by pulling out the tassel out of the leaf whorl.

- The entire operation should be done during morning 7.30 to 10.00 a.m.
- The tassel removed should not be carried in the entire openly & care should be taken to for disposing the tassel.
- The entire operation should be done in 8-10 days and daily.

#### 4. Male: female ratio

The male: female ratio depends on (a) pollen shedding potential and duration of male parent; (b) male: female synchrony :for better seed setting flowering of female should be earlier than male or male pollen dehiscence should coincide with female silking and (c) season. In general the male: female ratio should be 1:2 or 1:3 or 1:4.

#### 5. Field Inspection

Stages of crop inspection as follows:

**At the time of sowing purpose:** to monitor the land, isolation distance, planting ratio of male: female, proper sowing time, seed treatment etc.

**During pre flowering/vegetative stage purpose:** to verify the roguing and removal of off type plants (Photo 30 & 31 )

**During flowering stage purpose :** to check disease and pest infestation

**During post-flowering and pre-harvest stage purpose:** to remove the late and diseased plants

**Harvesting time purpose:** to see the proper time of harvesting



Inspection at vegetative stages



Inspection at pre-flowering stages

**Seed standards followed during seed production (Seed certification standards) of open- Pollinated Varieties, Synthetics and Composites, and hybrids ( given in table-11)**

Seed ears inspected after harvest shall not contain in excess of 1.0% and 0.50% for Opvs, Synthetics, composites and Hybrids respectively of off -type ears including the ears with off -coloured kernels.

Table-11

| Factor  | Standards for each class |           |
|---|--------------------------|-----------|
|   | Foundation               | Certified |
| Pure seed ( minimum)  | 98.0%                    | 98.0%     |
| Inert matter (maximum)  | 2.0%                     | 2.0%      |
| Other Crop seeds(maximum)   | 5/kg                     | 10/kg     |
| Other distinguishable varieties based on kernel colour and texture ( maximum) | 10/kg                    | 20/Kg     |
| Weed seeds (maximum)  | None                     | None      |
| Germination ( minimum)  | 90%                      | 90%       |
| Moisture  | 12.0%                    | 12.0%     |
| For vapour –proof containers ( maximum)                                       | 8.0%                     | 8.0%      |

### Hybrid seed production of OPVs, Synthetics, composites, and hybrids:

#### Seed production of open pollinated varieties (OPVs):

Open pollinated variety of maize should be maintained in an isolation from other field and roguing for plant characteristics should not be very strict as it may lead to random drift. Only off types, abnormal and disease and pest affected plants should be rogued. Population size of open pollinated variety should be maintained large with five thousand or more plants. Exact population size cannot be suggested accurately because it depends on the genetic makeup of a population. To make sure random mating in OPVs, 50 per cent of plants must be detasseled before pollination and it is practiced in every second row of field (is detasseled). Harvest and bulk the seed from detasseled plants only. Breeder, foundation and certified seed production should be in separate isolated large plots so that large scale random mating will take place and hence production of all genotypes in appropriate frequencies are possible.

#### I. Nucleus seed production of synthetics, Composites varieties, and Hybrids:

##### a. Synthetic varieties:

##### Synthesis of synthetic variety and nucleus seed production

Generally two methods can be followed to synthesis synthetic varieties.

- **Method I:** Equal number of seeds of each selected (based on general combining ability) line is mixed together and planted in an isolated plot under open pollination. Seed is harvested without selection for ear or plant types. The population raised from this seed is the synthetic-1 (syn-1)
- **Method II:** all possible crosses among the selected lines (based on general combining ability) are made in isolation. Equal numbers of seeds of each cross among the selected lines (if 8 lines (n) are there, a total of crosses will be  $n(n-1)/2$  i.e., twenty eight) are mixed together,

and planted in an isolated plot. The population derived from this composite seed is known as the synthetic-1 (syn-1)

The seed produced from syn-1 is used as nucleus seed which may be further used as a source seed for breeder seed production.

### **b. Composite varieties:**

#### **Synthesis of composites and nucleus seed production:**

Choice of the material that should enter into the synthesis of a composite shall depend upon the objective for which it is built. In general, it would be desirable to include open pollinated varieties, synthetics, and advanced generation hybrid sets. Which have wide genetic diversity. Divergence is indicated by geographic origin or pedigree of material.

Selected lines, based on their performance, are grown in ear to row system. Chain crosses may be made among selected lines and resultant seeds mixed together on equal number and grown in isolation. About three to five cycles of intermating are required to homogenize. At harvest five to six best ears from each collection are saved for next cycle. Different methods of crossing, half sibbing, full sibbing, backcross and chain crosses among the population have been employed to develop composite varieties.

Indian maize programme had released a number of composites such as kisan, jawahar, vikram, sona, vijay, amber, pusa chandan and pusa kundan.

### **c. Hybrids: Nucleus seed production /Maintenance of inbred lines of**

To preserve the performance and uniformity of a particular hybrid, the same pure breeding inbred lines must always be used. Variation in certain traits within same inbreds, from separate sources of maintenance, has been observed by many workers. Changes in breeding behaviour of an inbred line may be due to : (i) delayed segregation (ii) Mutation (iii) out crossing and (iv) mechanical mixture.

The maintenance may be done by planting ear to row or by mixing seeds from ears of individual plant of inbred line for increase and may be maintained by selfing or full sib-pollination by hands. Self-pollination is the process of applying pollen of a plant to its own silks. Sib-pollination is the process of applying pollen of a plant to the silks of a sister plant (plant of the same line). Sibbing tends to prevent excessive loss of vigour and selfing increases homozygosity and uniformity. Very often, in many maintenance programmes parental lines are maintained by alternate selfing and sibbing from one generation to the next. This alternate system of selfing one year and sibbing the next year is more advantageous (Fleming and Kozelnicky 1965). In sibbing or selfing population size must be appropriate. Population size should be little more in inbred lines developed by a limiting number of inbreeding (early generation inbred lines). In another case, if inbred line was a resultant of large number of inbreeding cycle (advance generation inbred lines) relatively smaller population size may be sufficient. In other words, population size during maintenance mainly depends on the degree of variation present in identified inbred line. However, such a programme should not be limited to only few years. Contamination by



foreign pollen must be avoided by maintaining desired isolation from all other maize plants.

### **Hand Pollination**

The maintenance or nucleus seed production of inbred lines generally involves hand pollinations. Cloudy, misty or rainy weather during flowering does not permit hand pollinations on a large scale. Therefore, either off-season sowing or sowing are adjusted to avoid undesirable weather conditions. As inbreds are poor in vigour, well-fertilized soil conditions and adequate spacing between plants are recommended.

Ears must be protected before silking with translucent paper bags called "silk bags" or isolator. The usual size of silk bags is 90×230 mm. The tassel bag may be used to cover the tassel of the same plant for selfing or other plant for sibbing. The tassel bag size should be 160 to 190 × 90 or 135 mm made out of brown paper. The date of bagging is marked in waterproof ink on the bag. This operation should be done before pollen shedding. For pollination, the paper bag isolator is removed from the ear and silk is shortened with pen-knife or scissors to about one cm. to facilitate even application of pollen the male plant is gently bent by holding it with the left hand in the flag leaf region and the tassel is tapped with the right hand to facilitate pollen shedding into the bag covering it. Crumple down the silk bag and slip the tassel bag over silk to be pollination and then pick up the bottom of the bag upwards and pollens are pumped around the silk and shake vigorously; this causes the pollen grains to fall upon the silks. Then the paper bag is folded at the bottom firmly around the stalk and fastened with a paper clip or stapler. This completes the pollination process. Care must be taken to avoid contamination with foreign pollen (Poehlman and Borthakur 1968).

## **II. Breeder seed production:**

### **a. Synthetics**

After a synthetic variety has been synthesized and nucleus seed is produced, the variety is maintained and multiplied by growing it in an isolated plot in subsequent years. Rouging may be done carefully and only undesirable (obvious off types) and diseased plants may be discarded, however, close selection on ear type is not advisable. Open pollination among all the plants is allowed sufficient random mating. The population derived from the seed Syn-1 is known as synthetic-2. Yield performance of synthetic-1 is better than synthetic-2 due to heterotic effects. Further, there will be no decline in yield of syn-2, syn-3, syn-4 and syn-5 due to zygotic equilibrium (Allard, 1960).

### **b. Composites**

Breeder seed of composite varieties may be increased in two stages as follow (Singh, 1987)

**Stage I:** five hundred or more half sibs should be carefully grown in isolation of the prescribed standard to produce the desired quantity of breeder seed for stage II. In half-sib block all female rows are detasseled, the seed for male rows is formed through a balanced composite.

**Stage II:** seed should be grown in isolation. The seed source for stage II shall be drawn from stage I. ten to fifteen thousands plant population should be maintained. In fact, this size of population is necessary to avoid inbreeding depression and genetic drift. Row length should be of 5-10 meter length and rejection intensity in any individual cycle should not exceed 10-15 per cent.

### c. Hybrids

Breeder seed production means the increase of each inbred seed stock obtained from nucleus seed in an isolated field. Field should be free from volunteer plants. Roughing should be employed strictly before pollen shedding. Roughing should continue in pre-flowering, flowering and post-flowering stages also. Breeder seed production involving long-term inbred lines, which have limited variation, need special attention, particularly at the time of roughing. In short-term inbred, roughing should not be very strict. An experienced plant breeder/seed technologist, particularly who is well conversant with the variation in the inbred line should be employed for seed production. Frequent multiplication of the breeder seed to the extent possible, should be avoided in order to avert any likely drift in the basic population, particularly in cases where lines have limited degree of inbreeding. It is recommended that each seed multiplication should be of an order of 5-10 quintals, which may be used for two-three years. Different classes of seeds for different types of hybrids mentioned as follows

#### Different classes of seeds for different types of hybrids

| Type of hybrid          | Classes of seeds |  |
|-------------------------|------------------|--|
| Single cross            | Breeder seed     | Multiplication of two inbreds  |
|                         | Foundation seed  | Multiplication of two inbreds  |
|                         | Certified seed   | Production of hybrid seed (A X B)  |
| Three-way crosses       | Breeder seed     | Multiplication of three inbred lines   |
|                         | Foundation seed  | Multiplication of one inbred (C) and production of one F <sub>1</sub> i.e., (A x B). |
|                         | Certified seed   | Production three-way hybrid (A x B) x C  |
| Double top cross hybrid | Breeder seed     | Multiplication of two inbred lines and OPV.  |
|                         | Foundation seed  | Multiplication of OPV and production of F <sub>1</sub> (A x B)                       |
|                         | Certified seed   | Production of three-way cross (A x B) x (OPV)  |

|              |                 |  |
|--------------|-----------------|--|
| Double cross | Breeder seed    | Multiplication of four inbreds                               |
|              | Foundation seed | Production of two F <sub>1</sub> , i.e., (A x B) and (C x D) |
|              | Certified seed  | Production of double cross i.e. (A x B)(C x D)               |

### Monitoring of breeder seed plots

The literary meaning of monitoring is "to observe critically". Generally four members team *i.e.* crop breeder, seed production breeder or maintainer, one officer from NSC and one officer from Seed Certification Agency where the seed is being produced visit the field at flowering time. The team evaluate the field for uniformity and varietal purity and submit their report on prescribed proforma.

Although there is no prescribed field standard for breeder seed production, however, monitoring team in their inspection report must mention that breeder seed crop under report is as pure as to guarantee that in subsequent generations *i.e.* certified foundation seed class shall conform to the prescribed standard of genetic purity.

## III. Foundation seed production

### a. synthetics

Before the seed is distributed to the farmers for commercial production, one or two cycles of seed multiplication should be done in an isolated field. By this time, population will reach to equilibrium. Selection or rouging should not be employed in a strict manner. If the production of seed is in sufficient quantity, then it may be distributed directly to the farmers.

### b. composites

The seed may be multiplied in prescribed isolated large size of plot following random mating. To avoid the undesirable out crossing or mechanical mixture, border rows may be raised from the same population. Selection pressure may be avoided, however, undesirable plant type and diseased plants must be discarded before pollen shedding. An intensive rouging may deviate the population from original genetic make up. Adequate plant populatin should be maintained.

### c. Hybrids

Foundation seed production is different for different types of hybrids as shown in Table 1. For foundation seed production breeder seed must be purchased from authentic source. As foundation seed is a certified class of seed, therefore, all precautions given by concerned certification agencies should be followed.

#### 1. Single-cross hybrids

In case of single-cross hybrid (two parent hybrid) two inbred lines are raised in separate isolated field. Roguing and other cultural practices are the same as described in breeder

seed production. Foundation seed of inbred lines will be used as source seed for the production of certified seed.

## **2. Three-way cross hybrids**

For three-way cross hybrid, two isolated fields are required. One for producing the F<sub>1</sub> seed of single-cross (A x B) and one for single inbred lines. In crossing plot, the usual planting pattern is two rows of the female inbred (A) to one row of male inbred (B). Detasseling of female 'A' line is necessary, and seed is saved only from female rows. The seed multiplication of third inbred line is done as usual in an isolated field. As soon as pollination is completed in the crossing block (A x B), male line (B) should be harvested and removed from the field.

## **3. Double-cross hybrids**

Foundation seed production of double cross hybrid means the production of two single cross involving four inbred lines (A, B, C and D). Two separate isolated plots are required to produce two single crosses i.e. A x B and C x D. Inbred A and C are detasseled when being crossed. However, breeder's advice must be obtained to identify female and male lines. Both crossed F<sub>1</sub> seeds should be clearly tagged with different colour labels indicating female and male F<sub>1</sub> seeds. This constitutes foundation seeds to produce certified (commercial) hybrid seeds. Selected fields for foundation seed (single cross) production need prescribed isolation distance (Table 2).

## **4. Double-top-cross hybrids**

Two inbred lines i.e. A, B, and one open pollinated or composite population are involved in the production of foundation seed for double-top-cross hybrids. Two isolated plots are required, one for producing F<sub>1</sub> seeds of single-cross between A and B inbred lines and another for open-pollinated variety (Table 1). In crossing plot, one inbred is to be used as female or seed parent and another one as male. Usual planting ratio is two rows of female inbred A to one row of male inbred B. As usual detasseling is necessary in female line. The open pollinated variety may be increased in another plot in isolation.

## **IV. certified seed production:**

### **a. synthetics**

Foundation seed may be increased by one more cycle of production as certified commercial seeds. Each cycle of multiplication must be in a large plot for providing chances of random mating and producing plants of all frequencies. The deterioration in the variety may occur due to deviation from random mating and this could overcome by reconstituting the synthetic as necessary from the original sources.

### **b. Composites.**

Seeds may further be increased in an isolated plot as done in foundation seed production.

## **C. Hybrids:**

### **1. Single-cross hybrids**

In certified seed production plot, planting ratio of 1: 1 or 3: 1 is usually followed. Adequacy of pollen production is a primary consideration in determining the ratio of female to male parent. Being poor vigour in inbred lines low female: male ratio (2: 1 or sometimes 1 : 1 also) is used in single-cross between two inbreds to assure adequate pollination. Female line is detasseled when being crossed. Once the vigorous inbreds are available, single crosses are natural choice (Vasal 1995).

### **2. Three-way-cross hybrids**

Under certified seed production, foundation seed of one single-cross (A x B), female parent and one inbred to be taken as male parent are planted with ratio 3 : 1 or 4 : 1. Generally, three-way cross hybrid is produced where three inbred lines, which combine well, are available but fourth suitable inbred is lacking, otherwise, double cross is more economical. Single-crosses (female) are more vigorous, early and tall whereas inbred line (male) short and poor which is the major problem in poor seed set. Detasseling of female (A x B) is necessary.

### **3. Double-Top-cross hybrid**

One single-cross and one open-pollinated variety (OPV) or composite variety, are involved in this system. A hybrid seed from single cross is taken as female parent and OPV as male parent. Higher planting ratio of 8 : 2 or 10 : 2 may be used in double top cross-hybrid seed production as OPV or composites are more efficient pollen parents. Plants may vary in degree of variation within a OPV (heterogeneous population) and it is a task of crop breeders to encompass the acceptable range in the description of OPV. Therefore strict roguing is avoided. However, obvious off-types, diseased and poor plants should be rogued out.

### **4. Double cross hybrid**

Double-cross hybrids are the most widely used type of hybrids. The foundation seed of two single-crosses (A x B and C x D) may be procured from authentic source. One of these single-crosses is to be used as the female parent and other single cross as male Parent. In fact, in each hybrid, male and female parent is pre-decided by the breeder. The single-cross A x B is detasseled when being crossed to C x D. The male parent is also a single cross hybrid and capable to shed more pollen for pollination. Therefore, the planting ratio between female and male parent may be increased to 8:2 or 10:2. The seed production of double-cross hybrid is quite cost effective. The double-cross hybrid crops are relatively more variable for plant characters than single-cross hybrid crop, which may be an advantage when crop is grown under adverse conditions. If necessary, a higher plant density of the male rows may be used and sowing the male rows could be undertaken on two dates in two very closely spaced adjacent rows to ensure better pollen availability for longer duration, (Singh 1987). Tunwar and Singh (1988) have prescribed the isolation distance requirements for certified seed production (Table 2).

### **Harvesting and threshing (Under the supervision of Certification agency staff)**

Male parent should be harvested first than the female and should be kept separately. Optimum moisture content in grain at harvesting should be around 20%. The harvested cobs should be spread evenly instead of making heap.

- The male lines should be cut immediately after the pollination is over
- Female lines should be harvested when completely dry and the ear heads should be sorted out
- The ear heads should be dried in threshing yard (up to 12%)
- May be dipped in melathion solution, dried and threshed
- Grading the seeds , discarding the small and malformed seeds

### **Shelling**

Shelling of female parent should be done earlier than male to avoid mechanical mixture. Shelling can be done manually or by power operated maize Sheller.

### **Seed processing, Storage and marketing**

All under size, broken, damaged etc seeds should be removed for maintaining the quality of seed. Seed drying should be done till the moisture content of the seed is reduced to 8 % and it should be kept in aerated jute bags. Seed should be stored at cool and dry place preferably in cold storage. Poor storage conditions will lead to loss of vigour and poor germination. Marketing should be done with specifications and standards.

### **Seed Yield**

The hybrid seed yield depends upon the type of hybrid seed produced e.g. Single cross hybrid mean the seed yield is low ( 8-10 qt/ac) as the parent is an inbred line and Hybrid seed yield will be more if it is three way cross hybrid of double cross hybrid (15 Q /Ac) as the female parent is already a F1 hybrid .

### **Conclusion**

To check the depleting ground water in country and to maintain the soil fertility by suggesting alternative remunerative crops to replace at least one third area from rice to other less water requiring but profitable crops like maize. Maize can solely contribute towards shifting area under rice cultivation and an action plan is going on with policy makers of our country. Production of hybrid maize seed is a unique and dynamic industry worldwide. Many steps are involved in the production, processing, and marketing chain. This work is often done under contract with private farmers, thus the selection, training, and collaboration with the best farmers is essential. Once the seed crop has been harvested it must be transported to the processing facility where it is de-husked, dried, sorted, cleaned, sized, treated, and packaged. The emphasize that Single Cross Hybrid breeding

program and to meet the nutritional security of maize eating population, the QPM would remain the focussed area of research and seed production programs would receive greater attention in this direction which will provide more employment, good yield and higher food grain production. So th joint venture of India and ASEAN countries will mutually benefitted through sharing of expertise in the filed oaf agriculture with special emphasize on maize seed production programme to feed their maize eating population.

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## Physiological-Harvestable Maturity Indices In Seed Crops

### Introduction

Plants, as sessile life forms, have evolved diverse mechanisms to circumvent unfavorable growth conditions, among them interruption of the life cycle is one of the most successful strategies. Spermatophytes, or seed plants, are characterized by the formation of the seed, a structure originated from the fertilized ovule that includes the embryo and other maternally derived tissues. Embryogenesis within the seed allows the entry into a quiescent state that represents an evolutionary advantage since it facilitates dispersal and resuming of growth under optimal environmental conditions. Seed formation is an intricate process that can be roughly divided into proper embryogenesis (cell division and morphogenesis), followed by a maturation phase, characterized by storage compound accumulation, acquisition of desiccation tolerance, growth arrest and the entry into a dormancy period of variable length that is broken upon germination (Harada, 1997). In essence, seed maturation involves all structures comprised within the seed, but predominantly the embryo and the endosperm originated by a double fertilization event. Maturation leads to a developmental end-point in the endosperm, whereas the embryo retains the regenerating capacity after germination. Despite these differences, both embryo and endosperm share many features concerning the physiological changes and underlying molecular mechanisms associated with maturation.

In this review, we will re-examine minor aspects of seed maturation as a physiological process opposed to germination and will focus on recent findings related to central transcriptional regulators that participate in gene expression programmes associated with embryo and endosperm maturation. In the past, the study of seed specific gene expression has led to the idea of different regulatory mechanisms in monocot- and dicotyledonous species. However, a closer watch uncovers a limited perception, probably sustained on a knowledge biased from the different prevailing tissues in the seed of the two phylogenetic groups. Current data, clearly points to the participation of similar and sometimes the same factors in both organs. Moreover, new roles, out of the seed, have been discovered for regulators considered to be seed-specific and new functions within the seed assigned to previously known regulators unrelated to this organ. Altogether, these findings are enabling to perceive seed maturation as an "intrusive phase" in the developing embryo, similar to other phases introduced in the course of evolution at different times and in different taxa to produce growth arrest in a reversible manner (Harada, 1999; Kaplan *et al.*, 1997). In addition, extensive studies of regulatory networks are facilitating the identification of master regulators, responsible for the "seed fate", which directly participate in transcriptional control during maturation or modulate the activities of other factors acting under their influence.

### Seed maturity

Seed maturity refers to the morphological, physical, and functional changes that occur in the seed from the time of fertilization until seeds are fully formed and ready for harvest. (Delouche, 1973).



Seed development is the period between fertilization and maximum fresh weight accumulation. And seed maturation begins at the end of seed development and is continues up to harvest (Abdul - Baki *et al.* 1973).

Seed maturation is the crucial and the most important factor that determines the seed quality. (Delouche *et al.* 1973).

#### Why to Study Seed Maturation

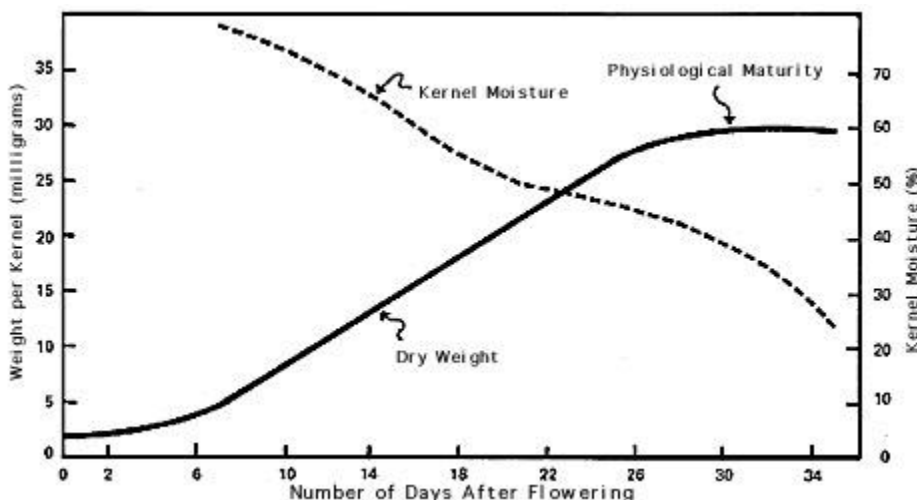
- It helps in prevention of seed deterioration by timely harvesting of seed.
- Harvesting can be done at earlier stage then the effective yield control can be formulated by preventing weed seed to attain full germination.
- It contributes considerably in saving time, money, and labor by avoiding unnecessary delay in harvesting.
- It helps in reducing seed loss due to insect's pests and non insect's pest such as rodents, birds and animals.
- It helps in escaping from the various types of diseases.
- It helps in reducing seed losses due to shattering.

#### Physiology of seed maturation

The maturation phase is started once the embryo and endosperm have completed the morphogenesis and patterning stages (Wobus *et al.*, 1999). This phase is characterised by a growth arrest, followed by the synthesis and accumulation of reserves, whose degradation upon germination will provide nutrients to the growing seedling before the photosynthetic capacity is fully acquired (Baud *et al.*, 2002). Early and mid phases of maturation are dominated by the action of ABA, initially synthesised in the maternal tissues and later on, although to a lower extent, in the embryo and endosperm (Nambara *et al.*, 2003). Transcription of major seed storage protein genes occurs mainly during this period. Subsequently, ABA levels decline and late maturation follows characterized by the synthesis of LEA (Late Embryogenesis Abundant) proteins, associated to the dehydration process and acquisition of desiccation tolerance. During this stage, accumulation of storage metabolites prevails in the form of carbohydrates (endosperm) or lipids (embryo), a quiescent state is accomplished and dormancy, the inability to grow under otherwise favourable conditions, can be established (Holdsworth *et al.*, 1999). Maturation is not an obligatory process and if embryos are removed from the seed and the ABA effects eliminated, they can proceed through the germination phase and develop into normal seedlings (see Figs. 1A, 4). In certain plants, like mangroves, embryogenesis proceeds directly to the seedling state. Similarly, so-called viviparous mutants in other plants display an analogous behavior. In addition to ABA, other hormones are important in seed development, like auxins, cytokinins and gibberellins (GAs). In particular, the synthesis and requirement of active GAs during the maturation phase have been demonstrated in elegant experiments in maize embryos (White *et al.*, 2000). It is now widely accepted that

maturation is not determined by ABA alone but instead by the ABA/GA balance. Exciting results on metabolites as signals, as well as metabolism and accumulation of nutrients during seed development have been extensively covered in recent publications (Baud *et al.*, 2002; Hills, 2004; Borisjuk *et al.*, 2004) and are outside the scope of this review.

### Physical and physiological changes during seed maturation



### Importance of understanding seed maturation

1. Unnecessary delay in harvesting seeds after they attain physiological maturity contributes considerably to deterioration.
2. Plant breeders can shorten the time required for growing crops by knowing the time of maximum germination.
3. Harvest can be done at an earlier date.
4. Effective weed control measures can be formulated by preventing weed seeds to attain full germination capacity.
5. Contribute considerably in saving time, labour and money by avoiding unnecessary delay in harvesting.
6. "Storing" of seeds commence in the field after the seed attain physiological maturity. Field deterioration of seeds can occur when subjected to adverse climatic conditions while still in the plant. Cottonseeds sprout in the boll, radicle growth of some grasses starts and legume seeds show water damage.

In general, seeds reach their peak germination and vigour at the time of maturation in the field. Once this peak is reached the seeds can only decrease in quality.

## Physiological maturity of seed

At this stage normally the seed has more moisture content. The seed crop can be harvested at high moisture content at physiological maturity, provided artificial facilities for drying are available; otherwise the crop has to be left on the field for natural drying till the seed moisture comes down to around 18-20 per cent for threshing. However, the harvesting maturity is a crop-specific character.

For seed threshing the moisture content of 18-20 per cent is suitable. Anything-above 18 per cent, the seed will be damaged. Increased amount of moisture content in seed amounts to increase in respiration, thereby seed deterioration increases at faster rate. However, the processing can be done at safer level of moisture content.

Seeds of most crop species mature when they attain maximum dry weight. Most of the seeds are physiologically mature at this point, but there are exceptions. However, after fertilization seed start developing, seed begin to increase steadily as a result of translocation of food reserves associated with rapid cell division and elongation. Physiological maturity is the days taken to attain maximum accumulation of dry matter, germination and vigour.

## Moisture Content (MC) at physiological maturity (PM)

|           | Moisture content per cent at |       |            |
|-----------|------------------------------|-------|------------|
|           | PM                           | HM    | Days to PM |
| Groundnut | 40-45                        | 35-40 | 65-70      |
| Rice      | 28                           | 18-20 | 25-30      |
| Sorghum   | 30                           | 20-25 | 30-35      |
| Corn      | 36-40                        | 20-25 | 50-60      |
| Cotton    | 50-55                        | 30-35 | -          |
| Chilly    | 50                           | 45-50 | -          |
| Soybean   | 20-25                        | 16-18 | 60-65      |

PM = Physiological maturity; HM = Harvesting maturity

## Percentage of food reserves in crop plants

|  | Starch | Protein | Fat |
|--|--------|---------|-----|
|--|--------|---------|-----|

|                       |       |       |       |
|-----------------------|-------|-------|-------|
| Cereals               | 70-75 | 10-13 | 2-8   |
| Oil seeds             | 5-20  | 20-40 | 40-50 |
| Pulses (beans & peas) | 50-55 | 22-25 | 5-10  |
| Soybean and cotton    | 15-26 | 37-39 | 17-33 |

Harvesting is the process of removal of entire plants or economic parts after maturity. The economic product may be grain, seed leaf, root or entire plant. The remaining portion of the stem that is left on the field after harvest is known as stubble.

### Types of maturity

There are two types of maturity

- ❖ Physiological maturity.
- ❖ Harvesting maturity.

Physiological maturity can be defined as the stage at which the seed reaches the maximum dry weight.

A seed is dried when it has dried out to moisture content in equilibrium with atmosphere this stage is known as harvesting maturity. Harvesting maturity varies with crop to crop; it depends on the use of the crop. Such as vegetable purpose, seed purpose etc.

The basic seed development pattern is most likely to be similar in most agricultural crops and horticultural crops. Although some variation may vary in levels of seed moisture and dry weight, duration of each stage which depends on genotype and environment factors. However the sequence may remain unaltered then the different stages.

### There are three stages or phases of seed maturation

- ✓ Phase 1 or lag phase.
- ✓ Phase 2 or food reserve or accumulation phase or linear phase.
- ✓ Phase 3 or ripening stage or desiccation stage.

#### Phase 1 or lag phase

- In this phase the seed growth is very rapid and marked at intensive cell multiplication.
- The moisture percent remains high and constant in this phase.
- This stage is important as it is the period when the frame work of future seed is being laid down.

#### Phase 2 or food reserve or accumulation phase

- In this phase there is a slow increase in the dry weight. Reaching maximum at the end of the phase.
- The amount of water changes very little but the percentage of water fall steadily.
- Seeds become viable early and acquired very rapidly.

- In this phase the substances which is served as food reserved such as protein sugar fat etc, are being transferred to the developing seed.

Plant nutrition is there fore is very important at this stage. Seed coat colour changes are the indices of approaching maturity which is gradually takes place during later half of the stage.

### **Phase 3 or ripening stage or desiccation stage**

- This stage last for 4-21 days depending up on the genotype and also the environmental conditions.
- During this stage the moisture content falls from 40% to the level in equilibrium with surrounding atmosphere (12-16%).
- During this stage the dry weight of the seed remains relatively constant and the seed is normally termed as ripe and ready for harvest and it is described as harvest maturity.

### **Common indices of maturity**

For most practical purposes a seed grower should consider a crop to be ripe when it is ready for harvest, when the crop is matured. But before the loss of seed due to shattering or shedding and decline the quality due to changes in various elements in environment.

The best point at which the seed should be harvested varies from area to area and year to year from farmer to farmer.

The common indices are as follows

- 1) Seed consistency:

Deciding harvest timing on seed consistency is offen hard to estimate the average

### **Effect of seed maturity on seed quality**

There are three important aspects of seed quality greatly affected by the different stages of seed maturation.

- 1) Viability.
- 2) Vigour.
- 3) Storage potential.

Viability: express as the ability or capacity of seed to germinate completely when it is placed on a substrata with optimum conditions for germination.

Many seeds harvested 10 days after pollination are viable and more than 90% of seeds harvested 15 days after pollination are able to germination.

Germination can does takes place very soon after the embryonic tissues have been found and before maturity are attained.

The immature seeds will not help in successful seed ling establishment.

### **Vigour**

It is the sum total of all those properties of seeds which determine the potential level of performance and activity of seed or the seed lot during germination and seedling emergence under varying conditions.

**Table. Criteria for harvesting of crops**

| <b>Crops</b>                   | <b>Maturity symptoms and criteria for harvesting</b>   |
|--------------------------------|--|
| <b>Rice</b>                    | <ol style="list-style-type: none"> <li>1. 32 days after flowering</li> <li>2. Green grains not more than four to nine per cent</li> <li>3. Percentage of milky grains less than one per cent</li> <li>4. Moisture content of grains less than 20 per cent</li> <li>5. 80 per cent panicles straw coloured and grains in lower portion of panicle in hard dough stage. At least five hills are to be studied at maturity</li> </ol> |
| <b>Sorghum</b>                 | <ol style="list-style-type: none"> <li>1. 40 days after flowering</li> <li>2. Grain moisture content less than 28 per cent</li> <li>3. Yellow coloured ears with hard grains</li> </ol>  |
| <b>Pearl Millet</b>            | 28 to 35 days after flowering<br>Compact ears, on pressing hard seeds come out   |
| <b>Finger millet</b>           | Brown coloured ears with hard grains   |
| <b>Maize</b>                   | <ol style="list-style-type: none"> <li>1. Less than 22 to 25 per cent moisture in grain</li> <li>2. Husk colour turns pale brown</li> <li>3. 25 to 30 days after tasseling</li> </ol>  |
| <b>Wheat</b>                   | About 15 per cent moisture in grain stage<br>Grains in hard dough<br>Yellowing of spikelets  |
| <b>Redgram</b>                 | <ol style="list-style-type: none"> <li>1. 35 – 40 days after flowering</li> <li>2. 80 – 85 per cent of pods turn brown</li> </ol>  |
| <b>Blackgram<br/>Greengram</b> | Pods turn brown or black with hard seeds inside pods   |
| <b>Groundnut</b>               | <ol style="list-style-type: none"> <li>1. Pods turn dark from light colour.</li> <li>2. Dark coloured patches inside the shell.</li> <li>3. Kernels red or pink</li> </ol>   |

|               |  |
|---------------|--|
|               | 4. On pressing the kernels, oil is observed on fingers |
| <b>Cotton</b> | Bolls fully opened                                     |

### Moisture content of grains for safe storage

| Crops   | Moisture Content (%) |
|---|----------------------|
| Paddy, raw rice   | 14                   |
| Parboiled rice  | 15                   |
| Wheat, barley, maize, sorghum, pearl millet, finger millet and pulses | 12                   |
| Groundnut pods, rape and mustard                                      | 6                    |

### Summary and conclusions

The following changes occur in seeds as they mature:

1. Moisture content decreases rather uniformly from 70-80 per cent to 15-20 per cent.
2. Dry weight increases to a maximum then may decrease slightly.
3. Seed size increases to a maximum then decreases somewhat as the seed dries.
4. A few seeds become capable of germination within a few days after fertilization; maximum germination is reached at a somewhat later date.
5. Seedling vigour increases as seed dry weight increases and reaches a maximum at the time of maximum dry weight (seed vigour is the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence).
6. In general, seeds reach their peak germination and vigour at the time of maturation in the field. Once this peak is reached the seeds can only decrease in quality.

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**Rice**



**Sorghum**



**Pearlmillet**



**Finger millet**



**Maize**



**Wheat**



**Redgram**



**Blackgram**



**Groundnut**



**Cotton**



**Table. Maturity indices, number and time of harvesting in vegetable crops**

| Seed crop             | Maturity indices   | Harvestings |            |  | Remarks                |
|-----------------------|--|-------------|------------|--|------------------------|
|                       |  | No.         | Time       |  |                        |
| <b>A. Dry seeds</b>   |  |             |            |  |                        |
| <b>Amaranthus</b>     | General brown yellowing of inflorescence indicate seed maturity  | 1 to 2      | Morning    | Prone to shattering  |                        |
| <b>Onion</b>          | seeds become black on ripening in silver coloured capsules. Ten percent heads expose black seeds       | 1 to 2      | Morning    | Prone shattering   | Jones nad Mann, 1963   |
| <b>Carrot Parsnip</b> | Secondary and 3rd order head turn brown  | 1 to 2      | Morning    | Shattering on delayed harvesting   | Gray, 1979             |
| <b>Spinach</b>        | Later ripening plants start to become yellow.  | 1           | Morning    | Shattering on delayed harvesting   | Parlevliet, 1968       |
| <b>Cole group</b>     | On ripening plants start dry out and become orange brown in colour. Oldest pod will become brown first | 2 to 3      | Morning    | Considerably shattering loss by birds. Strong tendency to siliqua shattering | Nieuwhof., 1969        |
| <b>Radish</b>         | Brown pods and parchment like when the seeds are near maturing   | 1           | Daytime    | Do not shatter easily  | Watts and george, 1957 |
| <b>Garden peas</b>    | Majority of pods have become parchment like  | 1           | During day | Do not shatter easily  | Biddle and king, 1977  |
| <b>Methi</b>          | Pods turns brown and leaves get dry  | 1           | Morning    | Delay in harvesting cause shattering   |                        |
| <b>Beans</b>          | Earliest pods dry and parchment like and remainder have turned yellow                                  | 1           | During day | Over maturity leads to shattering and  | Smith 1955             |

|   |   |        |            |   |                              |
|---|---|--------|------------|---|------------------------------|
|   |   |        |            | cotyledons cracking   |                              |
| <b>Broad Bean</b>   | Pods become relatively dry, sponginess and is usually preceded by a general blackening  | 1      | during day |   |                              |
| <b>Okra</b>   | Pods become gray or brown, hard according to cultivar   | 1 to 2 | during day | Sequential ripening of pods and a tendency to split on ripening           |                              |
| <b>B. Fleshy fruit which are dried before seed extraction</b> |   |        |            |   |                              |
| <b>Chillies</b>   | Turning of fruit colour green to red, yellow or brown.  | 2 to 3 | During day | dry methods of seed extraction  | Mehta and Ramakrishan, 1986. |
| <b>Bottle gourd</b>   | Rind becomes hard and colour  | 1      | during day |   |                              |
| <b>sponge gourd</b>   | Changes to light brown or yellow  |        |            |   |                              |
| <b>C. Wet fleshy fruits</b>                                   |   |        |            |   |                              |
| <b>Capsicum</b>   | Green coloured changes to red or yellow depending on variety  | 1 to 2 | during day | wet method of seed extraction   |                              |
| <b>Cucumber</b>   | fruit develops external ripening colour, stalk adjacent to the fruit withers. For confirming actual seed maturity, several fruits are cut longitudinally and mature seeds separate easily from the interior flesh | 1      | during day | Seed extraction is done by scooping, acid/alkali and fermentation methods | whitker and davis 1962       |
| <b>Watermelon</b>   | Tendrils withere on shoot bearing fruit. Skin colour undeseide the fruit surface resting on the soil is pale yellow. Dull sound on thum ping fruit  | 1 to 2 | Day time   |   |                              |

|                                       |   |        |          |   |           |
|---------------------------------------|---|--------|----------|---|-----------|
| <b>Muskmelon</b>                      | fruits tend to separate (full slip) from stem. In winter melons, seed maturity is indicated by rind colour change from green to yellow or yellow to white, blossom end of fruit softens, skin become waxy and its aroma increases. Easy separation by abscission layer. | 1 to 2 | Day time | Melon seeds not fermented   |           |
| <b>Squashes, pumpkins and Marrows</b> | Rind becomes hard and its colour changes from green to yellow, orange and yellow golden to straw colour.  | 1 to 2 | Day time |   |           |
| <b>Asparagus</b>                      | Berries become red or yellow and leaves turn brown  | 1      | Day time | select from healthy crop  |           |
| <b>Brinjal</b>                        | Turning normal fruits colour to red and softness of fruits  | 2 + 3  | Day time | Seed is extracted by fermentation acid/alkali or while juice/pulp separation, wet seed extraction | Rick 1978 |
| <b>Tomato</b>                         | Skin colour change to red and softness of fruits  | 2 to 3 | Day time |   |           |
| <b>Bitter Gourd</b>                   | Fruit and seed becomes red  | 1      | Day time | Hard seeds separated, washed  |           |
| <b>Summer squash</b>                  | fruits become hard, its colour deep yellow or red   | 1      | Day time |   |           |
| <b>Seed Potato</b>                    | Haulms get dry, droop down turn dark brown in colour  | 1      | Day time | Delay leads to spoilage of seed tubers.   |           |

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