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INTERNATIONAL TRAINING PROGRAMME
ON CAPACITY BUILDING
FOR OFFICIALS OF AARDO MEMBER COUNTRIES
on

# SEED PRODUCTION AND QUALITY EVALUATION

## January 14 – 28, 2018

**Compiled & Edited by:**

• D. Vijay • Monika A. Joshi • S.K. Lal • D.K. Yadava
• S.K. Jain • S.K. Yadav • Usha Rani Peddireddi

Sponsored by: Ministry of Rural Development, Government of India

**DIVISION OF SEED SCIENCE AND TECHNOLOGY**

ICAR - INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI – 110 012

# TRAINING MANUAL

## International Training

on

## SEED PRODUCTION AND QUALITY EVALUATION

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**2018**


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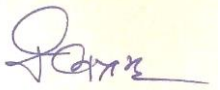
## FOREWORD

High-quality seed is a pre-requisite to achieve maximum outputs and good returns for farmers. Sowing good quality seeds leads to lower seed rate, better emergence, more uniformity, less replanting, and vigorous early growth, which helps in enhanced resistance to insects and diseases, decrease weeds and increase yield by 5–20 %. National Agricultural Research System along with Indian Seed Industry has played a significant role in seed production of many crops and ensuring the timely availability of seeds to the farmers has given a major impetus to generate their interest to adopt quality seed. Hence, adequate quantity of quality planting material at appropriate time and affordable cost needs to be made available to every farmer for bringing about radical changes in the agricultural scenario across the globe.

Significant advancements have been made in India with respect to production technologies and quality evaluation of seeds, which may not be in place at the same pace in many developing countries. I understand that with a view to share the knowledge of seed production and quality evaluation to the officials of African-Asian countries the Ministry of Rural Development, Government of India has sponsored this training program. The Division of Seed Science and Technology has covered all relevant aspects of seed production and quality evaluation, essential for managing standard seed production systems.

The training manual is an excellent source of information, which should be useful to seed technologists. I congratulate the team led by Dr. D. K. Yadava, coordinated by Dr. Sandeep Lal, Dr. Monika A. Joshi and Dr. Vijay D as well as other contributors of the manual.



**(K. V. PRABHU)**

## PREFACE

Seed is the essential component of agriculture and its quality is a crucial factor in realising the yield of any crop. Production of better quality seeds in an effective and efficient manner is a challenge for increasing food demand. Seed quality is a complex trait and novel research approaches to improve seed quality involve a combination of seed technology, genetics, and molecular biology. The progress in agriculture is therefore closely linked with the sustainable use of quality seeds of improved varieties and hybrids by farmers.

The knowledge upgradation through training is one of the essential components in the human resource development. With a view to impart knowledge of seed production and quality evaluation to the officials of African-Asian countries, the present African-Asian Rural Development Organisation (AARDO) sponsored training course for the participants from Taiwan (Republic of China), Namibia, Ghana, Nigeria, Mauritius, Sultanate of Oman, Morocco, Palestine, Sudan and Syria was planned. Since the Division of Seed Science & Technology, ICAR-IARI, New Delhi is recognised as the Centre of Excellence in seed research, teaching, extension and human resource development, the AARDO identified this division to conduct the present international training programme on "Seed production and Quality Evaluation" for exchange of knowledge as well as encouraging international collaborations and partnerships.

The technical programme comprised an exhaustive schedule covering the seed production and quality evaluation of major crops grown in the participants' respective countries. The hands' on experience during practical sessions and field visits was provided for effective learning. Lectures and interactive sessions were also arranged with eminent speakers from various disciplines along with the faculty of the Division.

With the objective to make available the information readily available for day-to-day use in performance of duties and as a reference to generate trained work force, all the lectures delivered were edited and compiled in the form of a manual. The present manual thus, covers complete and exhaustive information on all the aspects pertaining to Seed Production of important crops and Quality Evaluation.

We acknowledge and appreciate the wholehearted support of all the staff of Division of Seed Science & Technology and resource persons from ICAR institutes and private sector for their contribution in the success of this training. We extend our greetings and felicitations to all the participants.

***- Editors***

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# SEED INDUSTRY IN INDIA: PRESENT STATUS AND FUTURE PROSPECTS

**DK Yadava and KV Prabhu**

*ICAR-Indian Agricultural Research Institute, New Delhi-110 012*

Indian Agriculture has grown impressively and National Agricultural Research System with the Indian Seed Industry has played a critical role in its growth. It is well established universally that seed is the basic and most critical input for sustainable agriculture and quality seed is the pivotal input for sustained growth of agricultural sector and other inputs are contingent upon quality of seed for being optimally effective. Adequate quantity of quality planting material at appropriate time and affordable cost is to be made available to every farmer for bringing about radical changes in the agricultural scenario of our country. Importantly, this remains one of the most important challenges before the seed industry even today. But what we are witnessing today is a phenomenal progress which has occurred over a long period of time where both public and private sectors dealing in seeds have contributed enormously.

Harvesting the effect of post-independence efforts, greater emphasis was made on development of seed programmes starting with the First Five Year Plan (195 1-56) in independent India. The Grow More Food Enquiry Committee (1952) revealed that by and large seed of requisite purity was not available to the farmers. During the same year an Expert Standing Committee was established by the ICAR for formulating the concrete proposals for seed improvement programmes and as a result of these developments the schemes of seed multiplication and distribution came into existence in all the States of India. During the Second Five Year Plan (1956-61) the most significant milestone was establishment of the All India Coordinated Maize Programme. A special importance was given to the multiplication of nucleus seed to foundation seed. At each National Extension Service Block one seed farm and one seed store was proposed during this plan. Further a plan of setting up of 4328 farms of 10 hectares each was proposed of which 2551 farms were established during this plan. In addition, plan for setting up seed testing laboratories and cooperative stores were also drawn up. The first Indo-American Agricultural Production Team headed by Dr. Sherman E. Johnson of Ford Foundation suggested that village, block and district level extension workers should educate farmers for use of improved seeds; State Department of Agriculture should take up seed certification and cooperatives and private growers should take up the responsibility of seed supply. The team also recommended the setting up of seed testing laboratories in states, development of uniform Seed Certification Standards and Seed Law and creation of situations favourable for seed programmes. The second Indo-American Agricultural Production Team (1960) headed by Dr. Randhawa also endorsed the observations of first team. Prof. A.S. Carter, TCM expert visited India in 1959 and provided useful plans for

developing a sound seed programme in India. He also furnished a model state seed law which led to further developments on this matter. The various initiatives were reviewed by the various agencies like Progress Evaluation Organization (1960) and Seed Multiplication Team Review (1961) and they suggested various measures for the betterment of seed production, multiplication and quality control of seed of various crops. Release of four maize hybrids in 1961, led to the development of blueprints by the Ministry of Agriculture for rapid multiplication and distribution of these hybrids. Based on the recommendations of various high level review teams and keeping the urgency of quality seed production, distribution and seed law enforcement in view the Central Seed Corporation presently known as National Seed Corporation (NSC) was established in 1963 and the Indian Seed Act was enacted in 1966. A Seed Review Team was set by the Government of India in 1968 which visited several foreign countries and made far reaching recommendations like involvement of state agricultural universities in foundation seed production (Agarwal, 1997).

Uniform protocols and procedures for the seed quality evaluation and field standards for seed production were required at that stage. In this direction the lead was taken by the Indian Agricultural Research Institute, New Delhi where a separate section of seed testing was established in the erstwhile Division of Botany in 1961. Later on in 1968 it was upgraded to a full-fledged Division of Seed Technology to provide leadership in maintenance breeding, seed production, certification, quality evaluation, storage, physiology, pathology, variety identification, genetic purity evaluation and DUS testing for plant variety protection, development of seed quality assessment procedures and human resource development. The present nomenclature as Division of Seed Science and Technology was given in 1984.

The developments in the seed industry in India during last almost four decades are very significant. Launching of National Seed Project Phase-I during 1977-78 and further Phase II and III during 1978-79 and 1990-9 1, respectively was a major re-structuring of the seed industry by Government of India, which strengthened the seed infrastructure which proved to be the first turning point in shaping of an organized seed industry. Since the scheme All India Coordinated Research Project – National Seed Project (Crops) came into existence in 1979-80, revolution in seed production has occurred. The goal of AICRP- NSP (Crops) was to produce adequate quantity of breeder seed as per requirement of the country and conduct seed technological research to support quality seed production. The breeder seed is further multiplied to produce foundation and certified seeds by various agencies. ICAR launched the Mega Seed project in 10$^{th}$ Five year plan from 2005-06 and it is still continuing in 12$^{th}$ Five Year Plan under Consortium Research Platform. Directorate of Seed Research, Mau, UP has been entrusted with the responsibility of coordinating the activities under ICAR seed project “Seed Production in Agricultural crops”.

Likewise, introduction of New Seed Development Policy (1988-89) was another significant milestone in the Indian Seed Industry, which transformed the very character of the seed industry. It gave access to Indian farmers of the best seed and planting material available anywhere in the world. The policy stimulated appreciable investments by private individuals, Indian Corporate and Multinational Companies in the Indian seed sector with strong R&D base for product development in each of the seed companies with more emphasis on high value hybrids of cereals and vegetables and hi-tech products such as *Bt.* Cotton. The national economic liberalization regime introduced in the country in 1991, further accelerated the growth of the private seed industry in the country. The New Policy on Seed Development greatly liberalized import of vegetable and flower seeds in general and seeds of other commodities in a restricted manner and also encouraged multinational seed companies to enter the seed business. To begin with more than 24 companies initiated research and development activities and have made substantial commitments for investment on research and development in response to this policy initiative. The investments are expected to increase with increasing volumes of seeds of proprietary hybrids and preparedness of farmers to pay higher price for quality seed.

**Present status**

As a result of these interventions, the Indian seed industry has evolved by adopting and innovation scientific advancements in the areas of variety development and quality seed production. From an essentially non-existent seed industry five decades ago, it is truly an industry involving millions of farmer producers, hundreds of seed companies (both public and private), ably serving the cause of Indian agriculture. The Indian seed industry is a vibrant industry, consisting of players from the public (43%) and private (57%) sectors. The public sector in general is dealing with the high volume, low value crops, whereas, private seed sector is more engaged in low volume and high value seed crops. But with the licensing of large number of wheat, rice and maize varieties during last 2-3 years this notion is changing. Indian seed industry is the fifth largest seed consumer in the world, with 4.4% of global seed market, after U.S., China, France and Brazil. During last five years Indian seed industry is growing at a compound annual growth rate (CAGR) of 15% from Rs. 5000 crores in 2009-10 to Rs. 13500 crores (estimate) in 2014-15. This industry is undergoing wide ranging transformation including increased role of private sector, entries of multi-national companies, joint ventures of Indian companies with MNCs and consolidations. Indian seed industry is poised to grow at CAGR of 17% for next five years and will be ranked at number 2 or 3 in the Global Seed Business (Vasudevan, 2015).

The public sector comprises of one national level corporations viz. National Seed Corporation (NSC), 15 State Seed Corporations (SSCs), 24 Seed Certification

Agencies (SCAs), two Central Seed Testing and 122 State Seed Testing Laboratories (3 ISTA accredited and 20 have ISTA membership) which is providing requisite strength in serving the seed industry and farmers. The research and development in the public sector is dependent on public research under the aegis of the ICAR institutes and SAUs. SAUs and ICAR Institutes are also engaged in breeder seed production and also in production of foundation and certified/truthfully labelled seed of their varieties. Besides, seed is also produced by the farmers under Farmers' Participatory Programme of several Institutes and under Seed Village Programme of the Government of India. Following 34 agricultural universities and 22 ICAR institutes across the country are engaged in seed production activities. State Agricultural Universities are taking up breeder seed production involving its KVKs to bring seed revolution in the country. On the other hand, private sector is highly fragmented, with more than 600 players (including domestic and multinational companies) and the top 10 seed producers account for more than two-third of domestic market. Over the past two decades, private sector seed companies have collected germplasm and also built their R & D capabilities. Some of these have realised the importance of R & D and now spend about 5-10% of their sales on it. These players have developed many hybrids based on the local needs of the farmers and have been able to gain significant market shares.

The impact of Indian Seed industry on Indian Agriculture is clearly evidenced from the steep rise in production and availability of all classes of seed in the country. Over a period of eight years it has gone almost more than double. With availability of quality seeds we have been able to achieve a very good seed replacement rate in the major crops viz. wheat (32.55%), paddy (40.21%), maize (56.58%), sorghum (23.85%), pearl millet (60.40%), chickpea (9.35%), urdbean (34.41%), mungbean (29%), pigeonpea (22.16%), groundnut (22.5%), rapeseed-mustard (78.88%), soybean (52.75%), sunflower (32.47%) and jute (42.11%) in the country during 2012-13 (http://agricoop.nic.in/faq.html).

**Strength and the way forward**

The challenges before the world are food and nutritional security, energy, health, water and environment. These are the essentials for sustainability of life on the earth which will be further complicating due to climate change. Here seed is one of the key factors which can help in combating these challenges. There are many opportunities available in our system especially pertaining to seed and Indian Seed Industry, which can help a lot in facing these challenges. Some of these opportunities are as under:

- The competitive advantages that Indian agriculture processes are (a) Favorable agro-climatic zones (b) Large irrigated lands (c) Gap between present productivity and potential productivity and (d) Availability of skilled, educated, technical and scientific manpower. To leverage the global

competitive advantage, Indian agriculture needs intervention in the areas of policy, technology and market access.

- Public sector: A huge public sector paraphernalia including NSC, SSCs, SCAs, STLs, SAUs and ICAR institutes which are engaged in seed production, maintenance and quality evaluation is a great resource with the Indian seed industry.
- Private Sector: As mentioned above there are more than 600 seed companies including MNCs who are engaged in seed business is again a big asset to the Indian seed industry.
- Public Private Partnership: The very transparent, open and trustworthy PPP is existing in India which has led to the tremendous progress in Seed industry and it will go further for touching the new heights. The liberal government policies and a very conducive environment provided to the private seed companies have led to creation of trust which has been replicated rapidly. This also includes licensing of varieties of crops to private seed companies even to very small entrepreneurs who are actually the beginners at a very nominal fee with easily acceptable terms and conditions. It has led to attracting more than 300 small and big seed companies to get associated with the various ICAR Institutes and SAUs, which are proving very helpful in disseminating the good quality seeds of newly released varieties in a very short span of time. To cite an example, IARI has signed nonexclusive agreement with more than 200 seed companies and one NGO for seed production of different IARI released varieties of field and vegetable crops viz. Paddy (PRH- 10, Pusa Basmati 1121, Pusa 1460 and PB 1509), Wheat (HI 1563, HI 1544, HD 2967, HD 3059, HD 3086 and HD 3090), Mustard (Pusa Mustard 25, Pusa Mustard 26, Pusa Mustard 27 Pusa Mustard 28, Pusa Mustard 29 and Pusa Mustard 30), Maize (PEEHM 5), Cauliflower (Pusa Hybrid 1 and Pusa Kartik Shankar), Carrot (Pusa Rudhira), Cucumber (Pusa Sanjog) and Bitter gourd (gynoecious lines).
- Business Planning and Development (BPD) unit of IARI supported by National Agricultural Innovation Project, facilitates Public-Private Partnership (PPP) and extends support to industries and entrepreneurs with technologies, consultancies, project planning and business development. The start-up companies and perspective entrepreneurs can utilize the services of BPD for initiating their agricultural enterprises. They are enrolled as Corporate Members of IARI-BPD and BPD provides them an office space with moderate facilities of sitting, meeting client, internet and working laboratories. Besides, mentoring by IARI scientists and back up from laboratories and instrumentation facilities available in different divisions are also provided. This has helped in developing a large number of partnerships with several private companies for the commercialization of its technologies. IARI has also set up a farmers' producer company BEEJ

INDIA for enabling the technologies to reach farmers in the shortest time after their release.

**Policy interventions**

Undoubtedly Indian Seed Industry is in a very good shape. Private seed companies have a good share in seed supply of various crops. Still more seed production is required to increase the seed replacement rate of various crops. There are some issues where government policy based interventions are required. For the uninterrupted growth of Seed Industry in India *vis-à-vis* making it globally competitive the following issues needs to be addressed on priority:

**Seed replacement rate/ variety replacement rate:**

Except a few crops seed replacement rate in our country is very low. In few states it is less than 5% across the crops which are a very big challenge to the seed Industry and Indian agriculture. To overcome this situation there should be a rolling plan of the government where targets should be fixed to cover the area under quality seed. Moreover one more hurdle is dominance of very old varieties in the seed chain. There are examples in crops where very potential new varieties are available but major share of breeder seed in the crop is of very old varieties which also need the policy intervention. Likewise private seed sector can reach more rapidly to the unreached area but the cost of their seed is much higher in comparison to that of public sector seed. There should be some mechanism to have control over the cost of seed of private seed companies too so that the small farmer can also procure the seed and it will definitely help in enhancing the SRR. The approach of farmer first will have to apply.

**Genetically Modified Crops:**

Except Bt cotton no headway has been made in the development and commercial adoption of GM products in field and vegetable crops. Keeping the global trend of release of more and more GM products and challenge of meeting projected targets of agricultural production in the country, it is very crucial to think over this issue nationally and after complying with all bio-safety issues, research on GM crops should be made as an integral part of the crop improvement programme and accordingly their field and seed standards also need to be defined for facilitating their seed production. For making a headway the Biotechnology Regulatory Authority of India (BRAI) needs to be made operational, is urgently required and the regulatory system needs to be made highly efficient and full proof so that release process is not slowed down. Transparent procedures for testing, clearance and monitoring GM Crops will help in faster approval of potential materials.

**Use of molecular tools for varietal identity and genetic purity:**
Procedures and protocols laid out by ISTA are being followed for varietal identity and genetic purity in all seed testing labs in India. Molecular markers have now become an integral part of almost all the crop improvement programmes. The older methods for varietal purity and identity like Grow-Out-Test and other conventional tests are generally not that effective, time consuming and are influenced by environment. Use of molecular markers for varietal identity and genetic purity can be a very good option; more efforts are required for bringing it in practice.

**Organic seed production:**
The International Federation of Organic Agriculture Movements (IFOAM) is the worldwide umbrella organization for the organic agriculture movement, presently. The Government of India has implemented the National Programme for Organic Production (NPOP) for which APEDA is the nodal agency. The national programme involves the accreditation programme for Certification Bodies, standards for organic production, promotion of organic farming etc. The NPOP standards for production and accreditation system have been recognized by European Commission and USDA. Increasing demand of organic products has led to the seed production of such crops. Though, in a few states like Rajasthan the State Seed Certification agency has been re-designated to empower it for certification of organic seed production, yet no consensus seed and field standards for organic seed production have been laid out. Hence, if the organic seed production has to be to be done for producing the organic products as per international standards, the seed production issues of organic seed will have be addressed on priority. However, there are conflicting views regarding use of organically produced seed for organic production. This needs to be scientifically validated with respect to the residues in inorganically produced seed and carrying forward the residual effect of various chemicals used to the next generation in the production chain.

**Seed Bill:**
Regulatory mechanism for keeping the consistent supply of quality seed will be strengthened once the proposed Seed Bill 2004 is put in place. A long passage of time in implementing this bill is affecting the seed industry. It needs to be implemented for making the quality seed available to the farmers.

**Poor conversion ratio:**
There was a time when meeting breeder seed demand of various varieties was difficult. But now with the good facilitation through the various seed schemes like Mega Seed project, the indented quantity of breeder seed is being produced leaving few exceptions. In many crops it has been observed that the breeder seed indents are too high that only a very small proportion of that can cover the

entire area under a particular crop if it is truly converted to foundation and certified classes of seed. Over indenting for breeder seed and using that directly without putting the same in the seed chain puts and extra burden on the breeder seed producing Institutions. A control over this practice is required for maintaining the supply of breeder seed of high quality in different crops.

**Public Sector Seed Corporations:**

State Farms Corporation of India presently merged with National Seed Corporation have huge land area and very good infrastructure. The outcome of these giant seed production establishments is very poor. Special attention should be given to further strengthen these public sector institutions to get the maximum out of them. Once it is achieved the share of public sector in seed production programme may go much higher than the private seed sector. Naming a few like the three farms of almost 30000 ha at Suratgarh, Sardargarh and Jetsar in Sriganganagar district of Rajasthan. There are many more of this type of farms which can be the great asset to the public sector seed production programme.

**Global seed trade:**

Asian and African countries are now new areas for many seed companies for expanding their seed business. Asian countries like Bangladesh, Indonesia, Malaysia, Myanmar, Pakistan and Sri Lanka and African countries like Ethiopia, Ghana, Kenya, Nigeria, Sudan, Togo and Tanzania are mainly being selected. Due to limited availability of good quality seeds and adaptability of Indian material in these countries, there are ample scopes for the Indian seed companies. Huge investment is being made by large number of Indian companies in these countries for food grain production and plantation crops. For their successful production programme getting good quality seed is a major issue which can be meted out from India.

**Reaching the unreached:**

When we talk of global seed business in Africa and other Asian countries, we should take care of our own states where no attention has been given for making seeds of improved varieties available. These are the seven North Eastern States of our country where no seed corporation and seed certification agency is in existence. The vast areas of rice- Fallow lying vacant can be efficiently utilized under various *rabi* oilseeds and pulses once the good quality seed of such varieties is taken to these states. Special efforts are required to make the seed production of different varieties of various crops of that region which can make the dream of the "Second Green Revolution" true in the country. Here the various voluntary organization, self help group and small farmers can be associated, which will help both by improving the condition of small

entrepreneur as well making the good quality seed available to those remote or unreached areas.

**Climate smart seed production and storage facilities:**

Untimely rains and frequent fluctuations in temperature have made the conventional seed production sites unfit or less remunerative in terms of production and quality of seed. Current season's weather situation has created further panic as all the *rabi* crops have been severely affected. Abrupt rise and dip of temperatures during the crop season in the conventional seed production areas of various crops is again a serious issue which warns for searching the alternative safe sites for climate smart quality seed production. Likewise, the storage facilities also need to be climate smart for keeping the seed quality intact.

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http://seednet.gov.in/

# VARIETY DEVELOPMENT, EVALUATION AND RELEASE PROCEDURE IN INDIA

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In the post-Green Revolution era India has witnessed a spectacular advancement in agricultural production and productivity. Foodgrains production has increased more than five-fold increase, from 50 million tonnes in 1950-51 to 273.4 million tonnes in 2016-17 (3rd Advance estimates), and productivity increased by more than five times, from 522 kg/ha in 1950-51 to 2100 kg/ha in 2016-17. This transformation is attributed to the development and adoption of high-yielding varieties/hybrids in several crops. The pace with which the country has progressed in crop improvement programme would not have been possible without simultaneous evolution of institutional system for crop breeding research and seed production. Crop improvement research immediately after independence was augmented in phases through establishment of commodity-oriented National Institutes, National Research Centres and Project Directorates under the Indian Council of Agricultural Research. At present, there are one Deemed to be University, 19 Crop Research Institutes, 3 Project Directorates, two National Research Centres for improvement of different field crops and one Institute on germplasm conservation. Another major step of the ICAR was to launch crops oriented All- India Coordinated Crop Improvement Projects, starting with maize in 1957; followed by wheat and rice in mid -sixties. Presently there are 22 All-India Coordinated Crop Improvement Projects and ten All India Network Projects Projects devoted to research needs of different crops. In addition there are 44 State Agricultural Universities (SAU) and three Central Agricultural Universities (CAU) contributing towards the crop improvement research in the country.

A multi-disciplinary approach for varietal improvement and crop production-related problems; collective planning and testing; exchange of germplasm and breeding material; flexibility in operations, cutting- across administrative and other boundaries and linkages with International Research Centres are some of the characteristics features of the AICCIPs. Conceptually, this testing system facilitates generation of multilocation data within short period of few years. This unique model of multi-disciplinary approach based on multilocation testing facilitated rapid generation and identification of appropriate high- yielding varieties and development of improved package of practices for different agro-ecologies. Since 1965, 4477 high-yielding varieties/hybrids of field crops have been developed till May, 2017 combining desired levels of resistance to

biotic/abiotic stresses, adaptation to diverse agronomic variable and cropping systems and meeting prescribed quality standards have helped in revolutionizing crop production. The international nurseries and trials, being orgnaised worldwide by the CGIAR institutes, are basically modeled on the lines of AICCIPs.

The system of identification and release of varieties as well as production of nucleus/breeder seed and on farm verification trials are very well developed and standardized in crops like wheat and pearl millet which needs to be replicated in other crops also. Large-scale adoption of new breeding techniques such as marker-aided selection requires appropriate consideration in varietal testing procedures. Genetically engineered varieties have become a reality in several crops. Their development and introduction necessitate appropriate testing procedures for ensuring bio-safety. The development of export- oriented agricultural produce, including food-grains, is likely to increase considerably with globalization of economy and trade. Another important development is the increasing role of private sector involvement in crop breeding, seed production and supply. This demands creation of a reliable and transparent testing and evaluation system.

There are well defined procedures/guidelines for conducting All-India Coordinated Trials in a uniform way. Well defined procdures have been laidout for testing the improved material under All India Coordinated Crop Improvement Projects of different crops.

## I. SELF- POLLINATED CROPS

### A. Stages of Testing:

A three-tier system of multilocation evaluation spread over a minimum period of three years, involving following stages is a must for release of a variety or a hybrid.

(i) First year : Initial Varietal Trial (IVT)
(ii) Second year : Advanced Varietal Trial I (AVT-I)
(iii) Third year : Advanced Varietal Trial II (AVT-II)

### B. Mode of Induction of Test Entries:

(i) Test entries shall be included in the coordinated trials on the basis of the data generated in pre-coordinated testing (station and other trials). All data generated in the pre-coordinated tests on yield and other important agronomic attributes, reaction to insect-pests and diseases and relevant quality parameters are to be made available to the coordinator in support of inclusion of test entries in coordinated trials.

(ii) The entries shall be included on the basis of their superior

performance for yield and/or other desirable traits such as resistance/tolerance to key biotic/abiotic stresses, superior quality components, etc.

(iii) The entries must be characterized by a high degree of phenotypic uniformity and genotypic stability.

(iv) Germination and physical purity standards of seeds supplied for testing entry should conform to the minimum seed certification standards of that crop.

(v) The entries shall possess some distinct diagnostic features, making them different/identifiable from the varieties of common knowledge or use.

(vi) All information about the pedigree/parentage of the entry must be made available to the coordinator at the nomination stage itself by the sponsoring breeder.

(vii) Acceptance of entries for coordinated testing shall be subjected to the same system, irrespective of the method followed in the development of lines/strains.

(viii) Entries from the private organizations with established R & D units are inducted after discussion and approval by the annual workshop/group meeting of the respective crop.

(ix) The PD/PC units may nominate strains only for zones/situations for which well developed centres/programmes do not exist. They may concentrate on germplasm enhancement/pre-breeding activities to develop advanced generation/semi-finished elite materials and for introducing appropriate exotic materials for specific purposes and distribute these to cooperators/breeders to supplement their efforts.

**C. Evaluation of Test Entries:**

***a. Initial Varietal Trial (IVT)***

1. ***Constitution of trials:*** These trials would be constituted with new entries sponsored by cooperating breeders/institutions along with the specified number of check varieties, including latest identified/released varieties.

2. ***Number of entries:*** The number of entries (including checks) shall be limited to an extent where adoption of appropriate experimental design becomes otherwise difficult.

3. ***Checks:*** A minimum of three check varieties, comprising following shall be used.

(i) National check: A variety being grown over a wide range of environments/areas for a fairly long period of time (wherever available).

(ii) Zonal check: The latest released variety for the zone and the ecology.

(iii) Local check: A variety popularly grown in a given region/ecology/environment of the trial location.

(iv) Qualifying entries: Co-entries in the same trial and being tested for the

same number of years.

(v) Additional checks: May be included, wherever felt necessary.

(vi) The national (wherever available), zonal and local checks shall remain the same for a minimum period of three years to enable comparison with the same test entries.

**4. *Source and quality of seeds of entries:*** Genetically pure and true-to-type seeds, conforming to minimum seed certification standards, shall be used in trials. The agencies responsible for maintenance/multiplication, as indicated below, shall ensure that supplied seed quality conforms to the minimum seed certification standards.

(i) **Test entries:** The breeder/sponsoring institution/organization/company would supply seeds and would ensure that it conforms to germination and physical purity standards equivalent to the minimum seed certification standards of the crop.

(ii) **National/Long-term checks:** The required seed quantity has to be produced and arranged by the concerned coordinating centre/institution/organization.

(iii) **Zonal checks:** The seeds of the latest released variety are to be made available by the concerned breeder(s)/Institution/organization.

(iv) **Local checks:** Seeds of local check are to be supplied by the Zonal Coordinator/concerned breeder/institution/organization.

***5. Plot size, number of replications and field layout:***

(i) The trials shall be so laid out to enable detection of yield difference of 5-10% as significant at 5% level.

(ii) The experimental design, plot size and number of replications shall be decided in the workshop on the basis of experience gained from the past trials over years.

(iii) Plot size and number of replications shall be same uniformly at all the test locations/zone/ecology.

(iv) The experiment shall be laid out in a well leveled field of uniform fertility.

(v) All replications shall be accommodated in the same field.

***6. Management of the crop:***

(i) Cultural practices shall be clearly defined at the time of constitution of the trial, and be specified as decided by the workshop.

(ii) Appropriate range for date of sowing, seed rate, depth of sowing, row and plant spacings, fertilizer, water, weed, pests and disease management etc. shall be strictly adhered to at all the test sites as per the specified instructions supplied along with the trials.

***7. Test locations:***

(i) Same set of IVT for each specified situation shall be across all zones (where ever applicable) of the country in different regions, where the crop is predominantly grown.

(ii) The test centres shall be identified in the workshop. Test locations could be ICAR Institutes/SAUs/Main or Regional Research Centres/Zonal Research Centres/State Govt Centres.

(iii) Every effort should be made to have maximum number of test locations (minimum 3).

(iv) The trial entries shall be well spread over the cropping region.

***8. Monitoring of the trials:*** All the trials shall be monitored by a team of scientists to be deputed by the Project Director/Coordinator. The monitoring team shall have PD/PC/ZC/PI/Senior most member of team (Team Leader); Plant Breeder, Agronomist, Pathologist /Entomologist, Scientist of any other specified discipline (Members). The team shall visit the trial sites at around full flowering to maturity and record observations on quality of the trial conduct like plot uniformity within replications and test plots, crop stand, disease and insect-pest incidence, bird damage etc., and management as per specified norms and comment on the reliability of data likely to be generated. The monitoring team shall also indicate an overall estimate of yield of the trial on the basis of its observations, and give clear-cut recommendation whether the trial data should be accepted or rejected.

***9. Data to be generated:***

(i) Data on produce of economic importance, observations agronomic features like days to flowering and maturity, plant height, lodging, threshability; reaction to important diseases and insect-pests; easily measurable grain quality attributes such as grain colour, grain weight, grain appearance etc. are also recorded.

(ii) Additional data under artificial test conditions and hot spots for important diseases and insect-pests are also generated by the concerned discipline scientists by organizing separate set of screening nurseries/trials.

(iii) The Project Director/Coordinator supplies a set of data-books with details for recording various observations of each character along with the list of standard descriptors.

(iv) All cooperators need to record observations strictly according to guidelines provided and ensure the supply of one set of data- books to the coordinator by the specified date.

***10. Data processing:***

(i) All the data received at the coordination cell is be critically examined to decide suitability for inclusion for statistical processing. The trial data may be considered for discarding or acceptance for further processing on the basis of a) Recommendations of monitoring team; b) Suggestions by the Zonal Coordinator/concerned breeder; c) Deviation from specified range of sowing date, specified crop management practices for the trial such as fertilizer doses, irrigation levels etc.; d) Any other serious flaw in conduct of trial/data recording/reporting and e) Damage by animals/birds/natural calamity.

(ii) All the trials considered acceptable on the basis of above may be statistically analyzed and examined for the following before pooling of the results.

a) *General yield levels*: In the case of irrigated conditions, trials with extremely low yields (less than state/region/district average as per norms fixed by the workshop), normally attributable to poor crop management, or exceptionally high yields are to be discarded. Different criteria is adopted for trials under restrictive environments such as rainfed, salt-affected, waterlogged conditions etc.

b) *C.V. levels:* Irrigated trials showing highly erratic behaviour of genotypes over replications (resulting in non-acceptable high CV levels), arising from extremely heterogeneous fields, patchy plant stands, hazards like bird/animal/hail storm damages, may be discarded. In case of trials under rainfed/restrictive environments, all those showing significant genotypic differences and reasonable yield level of the checks should be considered. Trials with extremely low/negligible CV should be considered cautiously.

c) *Overall performance of checks:* Trials, where performance of the checks is low and unrepresentative of the general trial performance, is considered cautiously.

(iii) Data from all the trials qualifying on the above criterion is be pooled, analyzed for stability etc. and included in the result books.

(iv) The data on ancillary characters is compiled/ summarized appropriately and presented in the result books. Means and ranges may be given.

(v) Data generated in additional trials/nurseries by other disciplines is appropriately analyzed and presented in the result books.

(vi) Data once included in the data-books after scrutiny on the above basis is not be changed unilaterally by the PD/PC without the discussion in workshop/group meeting.

**11. *Norms for promotion of entries for testing in the second year:*** The promotion of entries from IVT to AVT would be strictly based on the overall performance/merit of the test entries. The following criteria would be followed.

a) Variety should qualify to minimum norms for reaction to pests and diseases, where required (*to be formulated by scientists of concerned discipline and approved by the workshop*). These norms should be reviewed from time to time by the workshop/group meeting as per the prevailing pest/disease scenario.

b) Outstanding performance for yield/main produce of economic importance (by a margin of 15 to 20%) over the best performing check without compromise on other important features.

OR

Significant superiority or 10% higher yield/main produce of economic importance over the best performing check and stable performance across locations in combination with specific favourable attributes such as high

degree of resistance/tolerance to diseases, insect-pests and other biotic/abiotic stresses relevant to the region along with acceptable produce quality characters.

OR

Yield/main produce of economic importance at par with the best performing check but significant superiority in some features of specific importance such as disease/insect-pest resistance/or some specific quality trait.

OR

Yield marginally lower than the best performing check but outstanding in one or more strategic features relevant to the crop such as extra earliness, specific industrial product property, export quality, nutritional superiority etc., which will result in higher cash returns per unit area to the cultivator.

**b. Advanced Varietal Trial-I (AVT-I)**

**1. *Constitution of trials:*** AVT-I shall be constituted separately for each recognized agro-ecological zone by the entries promoted from the IVT on the basis of the criteria specified earlier, the repeat entries from the previous year's AVT-I and check varieties.

**2. *Number of entries :*** The number of entries in AVT-1 shall normally not exceed 20. Higher number may be considered only as an exception.

**3. *Checks:*** A minimum number of three checks, comprising a National check (wherever available) and a Zonal check, which is the most recently released variety, along with the local check, as specified under IVT,s would be used. Additional checks such as the latest identified variety should also be included along with any other released varieties.

**4. *Source and quality of seeds:*** The source of seeds and seed quality standards for the test entries and checks would be the same as specified under the IVT earlier.

***5. Plot size, number of replications and field layout :*** AVT-I should be larger than IVT to make more realistic estimates of yield performance and to reduce inadequacies/errors of measurements inherent in small plots.

(i) The plot size for AVT-I shall be determined on the basis of the earlier experience in conducting AVT and on estimating sampling errors as well as on the basis of average yields recorded.

(ii) Design of the experiment to be followed shall be RCBD with 3-4 replications or any other suitable design so as to enable detection of minimum yield differences among entries as stated earlier.

***6. Management of the crop:*** This is same, as given under the IVT.

***7. Test locations:***

(i) The number of trial sites are much more than those for the IVT in a given zone. In addition to the IVT test centres, this trial should be conducted at other centres, which shall include zonal research stations of the State Agricultural Universities/Regional Research Centres of the ICAR

Institutes/Agriculture Colleges/KVKs/Agriculture Experimental centres of the Department of Agriculture Farms of the participating private sector organization including NGOs with adequate testing facilities.

(ii) The minimum number of test locations from which data of acceptable quality shall be generated should not be less than four per zone/ecology per season and preferably many more.

(iii) If for any reason, the number of locations from which data of acceptable quality are received fall less than four in a zone, the number of seasons/years of testing should be increased to maintain minimum test standards.

(iv) In case of critical/restrictive environments/difficult testing situations such as rainfed, salt-affected soils/waterlogged conditions etc., the number of test centres generating data of acceptable quality may be reduced to a minimum number of three per season/year, and number of testing years shall be increased.

**8.*Monitoring of the trials:*** The monitoring procedure should be the same as given for the IVT.

***9. Data to be generated:***

(i) In addition to yield and produce of economic importance data on plant stand, height, days to flowering, maturity and other easily measurable specified characters shall be recorded at all cooperating centres.

(ii) Data on disease and insect-pest resistance and other ancillary characters, are recorded only at the centres where facilities exist and is as specified by the workshop.

(iii) Data on quality parameters including biochemical and processing properties are generated from selected sites in specified laboratories.

***9a. Additional data to be generated at AVT-I stage***

(i) Data on field reaction to important diseases/insect-pests are recorded under artificial epiphytotic conditions by the concerned disciplines at appropriate locations including hot-spots. Data on pathotype/biotype variations, the level of virulence, etc. shall also be generated.

(ii) Research disciplines such as agronomy, physiology, soil science etc., as per the crop needs and seed availability are involved at the appropriate stage for evaluation of features relevant to the discipline. These discipline groups constitute and conduct separate set of trials for generation of relevant data.

(iii) Each entry are comprehensively described by the concerned breeder using standard descriptors for each crop [*in crops notified under PPV&FR, the descriptors specified by the authority for DUS testing must be included, which will facilitate registration*]. For this work, cooperation of seed technology research centres is being obtained and coordinated by Project Director/Coordinator.

***10. Data processing:***

(i) Procedure shall be same as specified for the IVT.
(ii) Promotion of entries to the AVT -II
The norms specified under the IVT shall be followed at this stage also.

**c. Advanced Varietal Trial-II (AVT-II)**
**Point No. 1 to 9 same as in AVT-I**
***9a. Additional data to be generated at AVT-II stage:***
In addition to the generation of data recorded in the previous trials and nurseries, more elaborate data need to be generated, which include the following.

(i) Response to the agronomic variables such as different dates of sowing, population densities in terms of spacing, levels of fertilization and irrigation etc., as recorded from the exclusive trials conducted at selected centres by the agronomists.
(ii) Response to popular and emerging new weedicides may also be studied.
(iii) Reaction to additional diseases and insect-pests of relatively lesser importance to the crop, including nematodes and bacteria, may be investigated and provided.
(iv) Attempt should be made to pinpoint genes responsible for resistance/tolerance in the new strains wherever possible.
(v) Intensive evaluation for specific quality parameters relevant to the crop, like oil recovery in oilseed crop/processing properties/actual chapatti-/noodle-/bread-making quality in wheat, cooking quality in rice/pulses, protein quality and micronutrients in food grain crops and any other quality trait important from nutritional aspects in concerned crops, should be worked out in laboratories where such facilities exist and are specified by the workshop.
(vi) Response to relevant abiotic stresses and other important characters are to be recorded with the help of concerned Projects Directors/Cooperators.
(vii) Additional information on farmer/consumer/market acceptance may also be generated through verification/on farm trials wherever possible.
(viii) Amenability of the variety to commonly used farm machinery such as seed drill, combine harvesting, picking etc. may also be recorded.

**10. *Data processing:*** Same as in the AVT-I.

**D. Procedure for Variety Identification**

Superior eligible test entries shall be identified based on the performance for specific crop zone(s) in the workshop/group meeting for presentation to the Central Sub-Committee on Crop Standards, Notification and Release of Crop Varieties. This shall be done by a committee called "Variety Identification Committee" constituted in advance of annual

workshop/group meeting with the approval of the Deputy Director General (Crop Science).

***a.Constitution of Variety Identification Committee:*** The Variety Identification Committee shall comprise of DDG (CS) or his nominee* (Chairman); Assistant Director General (Concerned Crop); Assistant Director General (Seeds); Project Director (DSR); Director of Research of Host Institute/SAU; Agriculture Commissioner (Department of Agriculture); One Director of Agriculture (State Government); One representative of Seed organization (NSC, SSC); Representative of crop-based processing/manufacturing industry; One representative of the private seed agency; Two eminent scientists (All members) and Project Director/Project Coordinator* (Member-Secretary)

*Essential members; a quorum of six members needs to be present

**b. Variety Identification Process**

***1. Eligibility for identification***

(i) The candidate variety will be eligible for identification on fulfilling the following minimum requirements.

(a) Three years yield data from coordinated trials under given ecology (rainfed/irrigated etc.) are required.

(b) At least two years data are a must on disease and pest reaction at the hotspot/artificial epiphytotic conditions under the coordinated project.

(c) At least one year data on agronomic performance with special reference to response to dates of sowing/planting/population density/fertilizer/irrigation levels is a must. These data will be only for the purpose of determining adaptive features of the variety.

(ii) Availability of enough pure seed is required for planting five hectares (excepting sugarcane). In case of non-availability in specified quantity, the identification process may be postponed to the later stage, at the most by one year.

(iii) Availability of pure/nucleus seed of high quality is desired with the breeder for producing quality seed in at least 0.5 hectares. The nucleus seed should have been produced as prescribed for each crop.

***2. Data Compilation***

The PD/PC Unit will provide the data of third year of test entries at least 20 days prior to workshop to enable the breeder for preparation of identification proposal and formal approval from the competent authority of the involved Institution(s).

(i) The data from three years of coordinated testing of the candidate variety shall be compiled by the concerned breeder/Institution/organization/company in the prescribed proforma**,** and should be submitted to PD/PC seven days prior to workshop.

***3. Presentation of proposal***

(i) Concerned breeder of the variety shall present the identification proposal

to the committee, highlighting characteristics of the candidate variety and other related issues.

(ii) The PIs may be asked to clarify specific points by the Committee.

(iii) In cases where PD/PC is also a contributing breeder of the proposed variety, PD/PC will not be associated in discussion and in decision-making concerning the specific variety.

(iv) The Committee shall state in brief the specific reasons for identification/re-testing/rejection of the proposed candidate variety.

**c. Variety Identification Norms:** The identification shall be on the basis of the following norms.

1. Significant superiority in yield/ in produce of economic importance over the best performing check (including qualifying checks) along with acceptable levels of other features.

   OR

   Yield/ produce of economic importance comparable to the best performing check and combining specific favourable attributes such as tolerance to biotic and abiotic stresses relevant to the region/agro-ecology along with other acceptable features — quality characters, suitability for specific cropping system of the region, etc.

   OR

   Yield marginally lower to the best performing check but is outstanding in one or more crucial traits such as specific product quality-nutritional superiority, industrial processing property, export quality etc., which will result in higher cash returns per unit area to cultivator/country.
2. The candidate entry should be uniform for important characters, like plant height, maturity, and particularly in respect of the distinguishing morphological characters in the region recommended for.
3. In case candidate variety qualified on the above characteristics is not distinct enough from the already released varieties on the basis of any of the morphological, or other diagnostic characteristics for which data are available, it should be made identifiable on the basis of biochemical/molecular markers before the final release.
4. The candidate variety must be stable for key trait(s) (such as resistance to a specific disease/insect pest), for which it has been identified for the particular region.

**d. Relaxation in Minimum Years of Testing for Identification:** One year relaxation in respect of the period of testing under the coordinated system can be considered by the Variety Identification Committee under the following situations.

(i) A genotype representing a recognized/recognizable major breakthrough in yield potential/in produce of main economic importance.

(ii) A genotype capable of minimizing heavy yield losses by containing major epidemic of any disease or insect-pest against which the existing varieties

are found vulnerable.

(iii) A genotype capable of providing safety against a widely occurring disease/insect-pest of major importance and causing economic losses on a large scale, and for which resistance is not available in the already released varieties.

(iv) A genotype possessing a special crucial feature, which is not available in the existing varieties, and which has wider implication on the agricultural exports/processing industries.

**e. Availability of Test Stock and Nucleus Seed:** Test stock seed (pure high quality seed produced by the concerned breeder/Institute) for sowing in five hectares for seed production/demonstrations/adaptive trials and nucleus seed for 0.50 hectare (except sugarcane) must be available at the time of making proposal for identification (for hilly regions and restrictive environments the above requirements can be suitably reduced).

**f. Molecular Fingerprinting of Identified Variety:** DNA profile or DNA fingerprinting of parental lines and hybrid along with the details of the molecular markers (where markers data-base is available in public domain) used may be generated after the identification of a particular variety before submission for release and notification proposal.

**E. Variety Release and Notification**

**i) Central release:** Once the variety/hybrid is identified, seeds of variety/hybrid/parental lines are to be deposited with the NBPGR for conservation in gene bank. After obtaining the acknowledgment with IC No. from the NBPGR, the release and notification proposal of the variety/hybrid needs to be submitted to the Central Sub-Committee on Crop Standards, Notification and Release of Varieties along with DNA fingerprinting data (*where ever available*) and good photographs of seed, single plant and of field view**.**

**ii) State release:** Genotypes tested in the AICCIP trials for at least one year or those which could not be identified on the zonal basis in the workshop but adequate information on disease and insect-pests reactions of them are available from the coordinated testing, can be proposed for identification for the concerned state after further yield evaluation trials with more number of sites within the state and with on-farm demonstrations. The variety has to be first cleared by the Institutional Variety Identification Committee of the concerned organization and then proposed to the State Seed Sub-Committee for release. Once the variety is released by the State Seed Sub-Committee, proposal is required to be submitted to the Central Sub-Committee on Crop Standards, Notification and Release of Varieties for its notificatio.

## II. CROSS-POLLINATED CROPS

### A. General Guidelines

The guidelines/stipulations for evaluation of varieties, hybrids, composites and synthetics as well as identification for release in the cross-pollinated crops shall remain same as in the case of self-pollinated crops excepting for the following.

### B. Evaluation of Test Entries

(i) There shall be two separate sets of trials one for open-pollinated varieties and the other for hybrids.

(ii) In the open-pollinated varietal trial, in addition to the latest released variety, the latest released hybrid for the respective region shall also be used as the zonal check.

(iii) In the Hybrid Evaluation Trial, the latest released hybrid and the open-pollinated variety shall form additional checks. In a situation where the candidate entry is the first-ever hybrid to be released, then open-pollinated variety shall be the check.

### C. Varietal Identification

In case of hybrids, an additional parameter to be considered for identification shall be the seed production potential of the hybrid combination.

## III. OFTEN-CROSS POLLINATED CROPS

For these, guidelines are same as for the cross- pollinated crops.

## IV. VEGETATIVELY PROPAGATED CROPS

For these, guidelines are same as for the self- pollinated crops. However in crops like sugarcane at least one year data on ratooning ability and ratoon yield would be essential and for varietal evaluation, besides public sector institutions, selected sugar factories may also be included as test locations.

## V. TESTING OF NEAR ISOGENIC LINES (NILs)

### A. Mode of Induction of NILs

Normally near isogenic lines developed by the breeders would be inducted into the testing system at the IVT level. However, specially developed NILs (using specially selected notified varieties) would be inducted into the first year AVT as per the following guidelines.

### B. Developing special NILs

The recurrent parent selected for the marker-assisted breeding programme should be a notified variety/parental line of a hybrid, which is widely cultivated

and accepted by farmers but has been suffering some production bottlenecks or lacking some traits, which when improved can add value to it.

(i) The selection of the recurrent parent for development of NILs under the AICCIP is to be decided by a Committee identified by Project Director/Project Coordinator for which approval of DDG (CS), ICAR, is also a must.

(ii) Recurrent parent needs to be selected with appropriate concurrence of the concerned breeder/organization/institution.

**C. Supported information to be provided**

(i) The breeder has to substantiate the proposed near isogenic lines (NILs) for its conformity to the parental variety with appropriate phenotypic data such as morphological and DUS data and molecular marker data before its nomination for trial.

(ii) The breeder has to provide details in terms of list of all the morphological/DUS characteristics for which the NIL is similar to and different from the recurrent parent at the time of the nomination of the NIL.

**D. Induction in the AVT-I**

(i) The breeder nominating entries under the AVT-I-NIL should enclose the list of molecular markers used for marker-assisted breeding, which includes those used for both foreground and background selections.

(ii) The NILs should have a minimum of 80% introgression as is estimated from parental polymorphic SSR markers through background selection or as decided by the crop breeding experts and genomic information available in a given crop.

(iii) A minimum of 6 parental polymorphic markers per chromosome should be used for this estimation.

(iv) There should be a minimum of two markers flanking the gene/QTL of interest if the gene/QTL is not in the telomeric or centromeric region. In the latter case, the marker identifying the gene/QTL should be reliably validated for detection of the gene/QTL with a minimum or no linkage drags.

(v) The breeder nominating the entry under the AVT-I-NIL trial should furnish details in a prescribed proforma at the time of nomination of the entry. A Committee constituted by the Project Director will examine the proposal and approve or reject nomination.

(vi) The NIL should have minimum two phenotypic characters (which can be easily assessed in the field) for the purpose of its identification and distinction from the parental variety. This will facilitate seed certification agency/seed law enforcement authority in the certification process.

Information on this should be supplied at the time of proposing for identification of the NIL.

**E. Evaluation of special NILs and generation of data**

(i) The NIL would be tested along with the recurrent parent as a check for two years to verify traits that are introgressed in the agroclimatic zone for which recurrent parent or original hybrid was notified earlier.

(ii) For yield purpose, the NIL would be compared with the recurrent parent variety for establishing its performance vis-à-vis parent variety.

(iii) For validation of the introgressed trait, the NIL would be tested with the donor parent for the target trait that has been transferred from the donor.

(iv) The NIL testing has to be carried out under the natural and artificial conditions (where target observation is for pest/disease/stress resistance) and other traits following standard experimental procedures and techniques along with the recurrent parent.

(v) Where the targeted trait is not disease/pest, the requirement of nomination for trial of having resistance/tolerance to disease/ pest is not a pre-requisite, since the recurrent parent is a variety already in a large-scale cultivation by the farmers.

**F. Monitoring of the trials**

(i) The monitoring of the trials is mandatory.

(ii) The Monitoring Committee should involve at least one specialist associated with the target trait along with the breeder(s) from the Institution(s) concerned.

(iii) Monitoring needs to be stringently carried out by the monitoring team both for the trait introgressed in the NILs and for the equivalence to the recurrent parent.

**References:**

Tandon J.P., Sharma S.P., Sandhu J.S., Yadava D.K., Prabhu K.V. and Yadav O.P. 2015. Guidelines for testing of crop varieties under the All- India Coordinated Crop Improvement Projects. Indian Council of Agricultural Research, Krishi Bhavan, New Delhi 110 001.

# DUS TESTING: PRINCIPLES AND PROCEDURES

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**Plant Breeders' Rights:** PBRs, like IPRs, are granted for a limited period, at the end of which varieties protected by them pass into the public domain. The rights are also subject to controls, in the public interest, against any possible abuse. The system of PVP based on UPOV convention has been developed specifically to cover plant varieties. The mission of UPOV is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants for the benefit of society. The UPOV Convention defines the basic concepts of plant variety protection that must be included in the domestic laws of the members of the Union leads, in itself to a great degree of harmony in those laws and in the practical operation of the protection systems.

**World Trade Organization & Plant Variety Protection:** WTO was established in 1995. WTO has more than half a dozen Inter-governmental agreements that affect agriculture. Agreement on Agriculture (AOA) is one of the provisions of Trade Related Aspects of Intellectual Property (TRIPs) agreements. TRIPS Agreement Article 27 (3) b requires member countries to protect plant varieties either by patent or by an effective '*sui generis system'* of protection or by a combination of both these systems. Since India is a signatory to WTO, it is obligatory to implement Plant Variety Protection in India. Looking in to the constitution of Indian farming, its need and wide consultation of planners, experts, policy makers, public and private institutions etc., the GOI has enacted the "Protection of Plant Varieties and Farmers Rights" (PPV&FR) Act, 2001, a '*sui generis system'*. The Act is unique in a sense that it protects rights of not only breeders but farmers and scientists as well. It affords reasonable protection for development of new varieties without compromising the rights of farmers.

***Establishment of the PPV&FR Authority:*** There is a provision of an independent and permanent body called PPV&FR Authority at National level vested with exclusive authority for implementation of the Act. It shall have a broad-based composition comprising scientists, state representatives, farmers/tribal, women's organization etc. The Authority consists of a Chairperson and fifteen members. Registrar General will be Member Secretary.

***Extent:*** All categories of plants except microorganisms.

***Period of Protection:*** The protection under this Act is granted for 18 years in case of trees and vines & in other cases, 15 years from the date of registration of the variety.

***Varieties that can be protected under PPV&FR Act***

- *Novel varieties*
- *Extant varieties*
- *Farmers' varieties*
- *EDV*
- *Transgenic varieties*

***Registrable Varieties:*** A new variety shall be registered under PVP Act if it conforms to the criteria of Novelty, Distinctiveness, Uniformity & Stability using its relevant characteristics (plant height, leaf shape, time of flowering etc.) by which it can be described as a variety in terms of the Act.

***Novelty:*** If at the date of filing of the application the propagating or harvested material of such variety has not been sold in India, earlier than one year. 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 application**.**

**DUS Testing**

Testing for distinctness, uniformity and stability is called as DUS testing. There are well defined UPOV guidelines for DUS testing of different crops which can be adopted as such or may be modified as per experience and need of a member country. Currently, DUS testing involves the comparison of new (candidate) variety with existing varieties for recording of a number of phenotypic characters or descriptors in tests in which new and existing varieties are grown side by side. All contemporary candidates should be compared.

**Guidelines for DUS Testing:** There are two types of DUS guidelines:

**General guidelines:** General guidelines provide general Principles and Procedures of DUS testing of crop varieties.

**Specific guidelines,** which deals with specific crops & sometimes may be specific for ploidy level like; cotton, wheat or specific types like pea- field pea, vegetable pea, garden pea.

**Duration of DUS tests:** Usually the DUS examination requires more than one independent growing cycles with reference to ecosystem of the variety for studying the consistency of results. The options for multiple growing cycles are:

- The candidate varieties are studied in a given location, over at least two successive seasons.
- For many crops, it is possible to complete two growing cycle in the same year. The two growing cycles should be independent of each other.

- For plants in green houses, provided the time between the sowing is not too short and the trial is randomized, at least partly, two growing cycles can overlap and still be compared as independent.
- For some crops such as fruit trees, the same plants are examined over successive years. The condition of independence of growing cycle is also satisfied in this case.
- In some circumstances authorities can allow one growing season. Such a possibility is mentioned in crop specific guidelines

**DUS Test Centres:** For selection of centres, following factors need to be considered:

1. Where the species can best display its characteristics.
2. Where there is least risk of damage from pests, diseases and weather;
3. Where most of the seed crop and main crops are grown; this gives DUS testing a link with the region where the characters will be expressed most often, through a high volume of seed certification and commercial crop production;
4. Where there is ease of breeders' access; breeders like to see how their varieties are performing in test and to discuss any problems.

However, in order to have better control and efficient data collection on DUS characters, less number of test locations is desirable.

In the initial years of DUS testing, two locations per agro-ecological zones are desirable, so that if test fails in one location, the data from another is available.

**DUS Test Designs**

The use of experimental design with respect to the number of growing cycles, lay out of the trial, number of plants to be examined and method of observation is largely determined by the number and nature of varieties to be examined in a particular trial. Because of the presence of only one treatment factor you can use RBD, CRBD, In-complete RBD etc.

**General DUS Test Guidelines**

**Characteristics to be used:** The characters listed in the Test Guidelines are those which are considered to be important for distinguishing one variety from another and which are, therefore, also important for the examination of homogeneity and stability. The characteristics must be capable of precise recognition and description. The tables of characteristics are not exhaustive but may be enlarged by further characteristics

Characteristics are subdivided in the Test Guidelines into their different states of expression, called in "states" and the wording of each state is followed by a "Note". For a better definition of the states of a characteristic in the Test Guidelines, example varieties are indicated whenever possible.

The characteristic used to distinguish varieties may be either qualitative or quantitative. "Qualitative characteristics" should be those which show discrete or discontinuous states while "Quantitative characteristics" are those which are measurable on a one dimensional scale and show continuous variation form one extreme to the other.
Characteristics which are assessed separately may subsequently be combined, for example the length/width ratio. In order to obtain comparable results in the various member States, the scope of the test (for example, size of plots, sample size, number of replications, duration of test, etc.) need to be fixed.
Qualitative characteristics are normally recorded visually, whereas quantitative characteristics can be measured; in many cases, however, a visual assessment of, if applicable, other sensory observations (for example, taste, smell) are sufficient, especially when measurements can only be made with considerable efforts.

**Selection of characteristics:** The characteristics should be readily identifiable characteristics associated with the crop, the amount of effort required to record those characters, and the value of those characters in distinguishing varieties. The characteristics must be important for the description of varieties and may be morphological, physiological, biochemical or of another nature. These characteristics should be important for DUS testing of varieties and not for their commercial value. The superiority of usefulness of a variety is not criterion for protection. Basic requirement of characteristics for DUS Testing:

a) Capable of precise definition.
b) Produce consistence and repeatable results.
c) Allow uniformity requirements to be fulfilled.
d) Clearly defined in the observation and evaluation of results.
e) Allow a clear differentiation among the varieties.
f) Least susceptible to environment influence.

Characteristics like, disease resistance, chemical resistance (herbicide) as well as characteristics based on chemical constituents may be included provided they are tested.

**Grouping of Varieties:** It will be very difficult task to assess the candidate variety for distinctness against all the varieties in reference collection or in common knowledge. The selection of varieties of to be grown in the trial along with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness is aided by the use of grouping characteristics.

**Grouping characters:** Grouping characters facilitate grouping of varieties in few categories. Characters for grouping are those which are known from experience not to vary or to vary only slightly within a variety. Which in their

various states of expression are fairly evenly distributed throughout the collection. For example in wheat, grouping characters are:

- Ear colour
- Time to heading
- Auricle pigmentation
- Plant height

**Recording of observations on characteristics:** Four types of recoding are used:
VG : Visual assessment by a single observation of a group of plants or parts of plants.
VS : Visual assessment by observation of individual plants or parts of plants.
MG : Measurement by a single observation of a group of plants or parts of plants.
MS : Measurement of a number of individual plants or parts of plants.

**Asterisked characteristics:** These are characteristics that the experts consider important for DUS testing. So these should be used as a matter of routine for all varieties in every growing period. Such characteristics should always be included in variety description except when the regional environmental conditions render it impossible.
(a)They are marked with asterisk (*) in the test guidelines. A characteristic should only receive an asterisk status if it is (a) important for description.
(b) If it is needed as a minimum information for the exchange of information on the variety.
(c) All experts agree to asterisk at least.
(d) For a pest or disease resistance characteristics, it has only "absent or present" states;

**Recording of colour characteristics:** Examiner of DUS test is usually required to record the observations on colour characteristics of plant such as seedling, leaf, petal, seed etc. Since each colour manifests several shades, recording of precise colour and shade becomes confusing. It is therefore, recommended that Royal Horticultural Society (RHS) colour charts should be used while recording colour characteristics.

**Special characteristics:** If, the DUS based on morphological characteristics fails to establish distinctiveness of candidate variety & breeder makes a request, the special characters may be allowed for DUS like;

- Characteristics expressed in response to certain external factor viz; disease resistance, chemical resistance & Characteristics based on chemical constituents may be used for DUS testing provided these characteristics

are well defined and an appropriate method is available to ensure consistency in examination.

- Combined characteristics: Characteristics that are assessed separately, but have biological connection, may subsequently be combined like ratio of length to width. Combined characteristics must be examined for distinctness. Uniformity and stability to the same extent as other characteristics.

**Sample size for DUS Testing:** The UPOV recommendations to put 60 plants (3times 20) into a DUS trial is not a general rule. The question is what is the optimal sample size in DUS testing for specific crop over all characteristics? For qualitative characteristics distinctness procedures are not the basis to determine the optimal sample size up to now. However, for uniformity point of view, the optimal sample size can be calculated. Maximum of determent sample size is from the statistical point of view the options. The sample size depends on a number of factors like;

- Precision at the stage of individual single plants (within plots)
- Precision at the stage of replication (over the plots)
- Precision for years or cycles (over the years or cycle)
- Uniformity of a variety within the species.
- Type of characteristics in respect of variability within the variety over the plants and over the year /cycle.

**Technical questionnaire:** Technical questionnaire is a part of guidelines, which deals with the following information with regard to specific crop;

1. Genera / Species
2. Applicant (Name and Address)
3. Proposed denomination of the variety as given in Form No 1:
4. Information on origin, maintenance and reproduction of the variety
5. Characteristics of the candidate variety to be given (the number in Note column refers to the different states of expression of given characteristic described in column 3. Tick out within the brackets, which best corresponds to the character expression of the candidate variety):
6. Differences of candidate variety from similar varieties:
7. Additional information, if any, which may help to distinguish the variety

**Specific DUS Test Guidelines**

For specific requirements, one has to decide about seed quantity, its quality, method of submission, test plot design, methods & observations, grouping characters, table of characteristics, explanation on table of characteristics, TQ etc, plot size, number of rows, row length, row to row/ plant to plant distance, expected plants/replication, number of replications, number of plants/parts of plants to be observed.

**Table of characteristics:** The major columns in the table are characteristics, different states of each characteristic, notes for different states of characteristics, name of example varieties, stage of taking observation for a particular character, type of observation– VS, VG, MS, MG. *****The characteristics which are marked with asterisk are important characteristics for DUS testing and should be used for all varieties in every growing period and included in variety description. These are characteristics for which diagrams or methodology is required in the guidelines like;
Phenol reaction in wheat: Grains are soaked in 1.5 percent aqueous phenol solution for 24 hours, drained and air-dried. Hull colour is then recorded unstained and stained. (Chang T.T. & E.A. Bardenas, 1965).

**Seed submission and standards:** The applicant is required to deposit prescribed quantity of seed/planting material. The seed material should not be subjected to any chemical or bio-physical treatment. The applicant shall have to ensure its quality like germination.

**Example varieties:** Wherever possible in the table of characteristics, example varieties are indicated against the state of expression of different characteristics Example varieties are included in the test conducted for testing candidate varieties for DUS. These are used only as a help. The testing would become too difficult if example varieties have to be used for each state of expression of all the characteristics of a species out of the example varieties indicated in the National Guidelines. The authorities responsible for testing will choose the ones, which they consider most appropriate for the solution of a given problem.

**Procedures for DUS Testing**
**Application (Technical Questionnaire):** The breeder seeking protection for his new variety is required to submit an application form and a detailed technical questionnaire accompanied by a statement containing a brief description of the variety bringing out its characteristics of novelty, distinctiveness, uniformity and stability as required for registration

Although the breeder is required to describe the characteristics of his new variety to the best of his ability in the questionnaire, his description is not regarded as defining the limits of his claim to registration. Its main function in practice is to guide the authorities in carrying out trials on the living plant material, including the selection of suitable control varieties for the purpose of comparison

**Preparation for PPV&FR Act implementation in India:** We started the work in this direction much before the GOI enacted the "Protection of Plant Varieties and Farmers' Rights" (PPV&FR) Act, in 2001. In 1990s, while discussions were going on at national & international level for PVP, the preparations started at

ICAR under the dynamic & foresighted leadership of Dr. R. S. Paroda, than DG, ICAR & Dr. Mangala Rai, DDG (CS), ICAR.

We in NSP (Crops) started work on characterization of varieties vigorously in 1998 under the leadership of Dr. R. K. Chowdhury, PC NSP at 8 centres of NSP on 14 crops (Wheat, rice, maize, sorghum, pearl millet, gram, pigeon pea, mung, urd, groundnut, soybean, sunflower, castor & cotton) with the objectives to generate the data base of varieties of these crops which may be used for PVP. More than 1000 varieties were characterized in this project as per DUS characteristics & later on harmonized it with UPOV. This provided solid foundation for data base of reference varieties for DUS testing.

The second major step was the decision of ICAR to constitute a 'Core Group' under the Chairmanship of Dr. S. P. Sharma/Dr. R. K. Chowdhury for the development of DUS Test Guidelines and other advisory role to the ICAR thinking that the Protection of Plant Varieties & Farmers' Rights Authority may not have infrastructure for DUS Testing & therefore, ultimately the ICAR may be given this responsibility.

The Govt. of India had selected/priortized 35 crops for PPV&FR Act to start with. Out of these, 25 are field crops, 8 vegetable crops and 2 flower crops as detailed below:

Cereals- Wheat, rice, maize, sorghum, pearl millet,
Pulses- Gram, pigeon pea, mung, urd, lentil, pea & rajmash.
Oilseeds- Groundnut, soybean, sunflower, castor, safflower, sesame, linseed & rapeseed mustard.
Fibre crops- Cotton & Jute.
Sugar crops- Sugarcane
Forage crops- Berseem (Egyptian clover) & lucerne (*Medicago sativa*)
Vegetables- Potato, tomato, brinjal, okra, cauliflower, cabbage, onion & garlic.
Flowers- Rose & Chrysanthemum.

We, in India, have decided to develop our own guidelines named as 'National Test Guidelines' for DUS of different crops. The National Core Committee on DUS Test Guidelines constituted by ICAR prepared the test guidelines for individual crops with close cooperation of PCs/PDs /Directors /Institutes & expert scientists & named them as 'National Test Guidelines' for DUS of different crops. These National Test Guidelines for DUS of different crops have been taken basically from UPOV Guidelines except some change/improvement based on our requirement /experience/ expertise. After joining of Dr. S. Nagarajan as first Chairperson of PPV&FRA in October 2005, the PPV&FR Authority started functioning. The Authority constituted 3 Task Forces for evaluation of DUS test Guidelines developed by Core Group. Task Force 1/2006 was constituted for DUS guidelines of 12 crops of cereals and pulses namely; rice, wheat maize, pearl millet, sorghum, chickpea, pigeon pea, mung bean, urd

bean, lentil, field pea & rajmash, development of harmonized descriptors of plant varieties and the Application forms for plant variety registration under the Chairmanship of renowned scientist and administrator Dr. M.V. Rao with 10 other members of eminence, Task Force 2 was constituted under the Chairmanship of renowned scientist and administrator Dr. Y. S. Nerker with other members of eminence for the rest 13 field crops (Oilseeds & other field crops) & Task Force 3 was constituted under the Chairmanship of renowned scientist and administrator Dr. G. K. Kaul with other members of eminence for vegetables and flower crops. These Task Forces screened/evaluated the guidelines developed by 'Core Group' and recommended to PPV&FR Authority for final approval & notification. These guidelines were later on notified & published by PPV & FR Authority. Simultaneously, the Authority published the various fees for conduct of DUS tests, annual fee, application handling charges, registration.

We have decided that New Candidate Variety will be tested in replicated trial with suitable reference varieties over 2 similar growing seasons for recording of a number of phenotypic characteristics at 2 locations.

Usually the New Candidate Variety will be tested in replicated trial with suitable reference varieties at 2 locations. Out of these 2 centres, one will be primary centre of which data will be used for DUS examination. The second centre will be covering centre in case the trial at primary centre is vitiated due to some unavoidable reasons like natural calamities. We have also decided that the primary centre will be crop based institute where we expect best facilities & expertise for DUS examination.

In the first phase, the PPV & FRA decided to implement plant variety protection for 12 crops (rice, wheat, sorghum, pearl millet, maize, chickpea, pigeon pea, mung, urd, lentil, field pea and rajmash). These crops were notified by the PPV&FRA. Sh. Sharad Pawar, Hon'able Union Minister of Agriculture & Consumer Affairs, Food and Public Distribution, Government of India officially launched the registration of plant varieties for protection on Feb 20, 2007. Thus the registration of new and extant varieties started w.e.f. May 21, 2007.

**DUS Testing to be done for:**

**New varieties-** Replicated trial with suitable reference varieties over 2 similar growing seasons for recording of a number of phenotypic characters.

**Varieties of Common Knowledge-** Similar tests but for 1 season.

**Farmers' varieties -** Paired row tests for 1 season at 2 locations.

**Trees & vines-** Field & multi-location test for 2 similar seasons & special test lab based. Also option for on-farm test sites - 2 seasons.

**Special tests:** If field test fails to demonstrate the distinctiveness than we will do Special tests which may be physical, biochemical, molecular, response & organo-leptic parameters.

**DUS Trials**

The New Variety: shall be tested in replicated trial with suitable reference varieties over 2 similar growing seasons for recording of a number of phenotypic characteristics. DUS trials shall be monitored by a Monitoring Team. The applicant may also join the team by paying prescribed fee.

**Extant Notified Varieties:** The Extant Varieties Recommendation Committee (EVRC) comprising of 7 members was been constituted by the PPV&FRA to advise & provide the guidance to the Authority for registration of extant varieties under the PPV&FR Act 2001. The extant varieties recommended by the EVRC are further processed for registration by the Authority.

**Requirements for DUS Testing:** We need to have DUS Test Guidelines, Data base of extant /notified varieties, notified DUS test centres, Pure seed of extant/notified varieties and their safe storage, Required agronomic practices, Soft ware for data base/analysis, notified Special Tests and vigorous Awareness/capacity building programme.

**Requirements for the application:** Applicants are required to provide information on i) Origin of the genetic/parental material for the development of the variety & it is lawfully acquired, ii) Not using the Genetic Use Restriction Technology or "Terminator Technology" /"GURT". (Affidavit required), iii) Complete passport data including that of parental lines and geographical location of the parental line, iv) Contribution of any community/others in the breeding/evolution or development of the variety,
v) Photographs of the distinct characters. vi) Authorization by the breeder in case of an institutional application(PV-1) & proof of right to make application (PV-2). Applications in English/Hindi & in triplicate shall be submitted to Registrar, with registration fee, prescribed seed and DUS test fee. For farmers' variety, application is required to be endorsed by concerned Chairperson or Secretary of the Biodiversity Management Committee/ District Agricultural Officer/ District Tribal Development Officer or Director of Research (State Agricultural University).

**Seed submission and standards:** The applicant is required to deposit prescribed quantity of seed/ planting material as per Sec 27. In case of extant varieties, the applicant shall have to submit 2/10 quantity specified for new varieties and for Farmer's verities as well as verity of common knowledge, ½ the quantity of seed material/planting material specified for new varieties of same crop species. The seed material should not be subjected to any chemical or bio-physical treatment. The applicant shall have to submit along with the seed a certified data on germination test made not more than one month prior to the date of submission from an accredited laboratory.

**Procedure for examining distinctiveness**

**Main steps:** In the office, we study the technical questionnaire (Full information on the origin and structure of variety, correct description of all requested characteristics, reference to well known varieties, any additional information on a specific trait of the variety, possible use of a morphological distance combining the TQ characteristics, pre-distinctiveness, use of grouping characteristics & selection of similar varieties. Depending on the species, possibility to consider firstly the reference varieties which are largely used or known as having good performance in the area where the application is made.

**First growing cycle:** First official description of the variety based on DUS test guidelines, we conduct:

- Good trials with two locations when possible
- Take observation of any particularity of the variety along with the cycle
- Possible use of morphological distance
- Rejection (of new first cycle) for any variety with a wrong TQ description
- Contact with applicant to get any information on the distinctiveness from the closest variety.

In the office, study the first official description for distinctiveness like;

- Comparison with the reference varieties grown in the same cycle.
- Not grown in the same cycle
- Elimination of the clearly distinctive varieties
- Selection of the closest varieties
- Organization of the next cycle lay-out

**Second growing cycle**

**Description:** Second official description as for the first cycle plus any additional characteristics mentioned by the applicant.

Distinctiveness: Direct comparison of the candidate and the closest varieties

Conditions with possible use of specific lay-out to compare the varieties (side by side, row plots etc.), possible use of panel of experts and/or visit of the trials by the applicant.

In the Office, see if the variety is clearly distinctive (plus uniformity and stability). In case, the report is positive & consistent differences among 2 cycles, the variety should be accepted for registration.

If in the final description, the variety is not clearly distinct from one or several reference varieties with no difference observed and no claim from the applicant, the decision would be rejection.

**Third growing cycle:** With no difference observed and claim from the applicant with additional reliable information or with a set of small differences but not consistent over the two cycles and experts convinced that the candidate variety is original:

* If supporting evidence
* If no supporting evidence

Accept for third growing cycle.

**Third growing season**

**Distinctness:** Direct comparison of the candidate and the similar reference varieties. Conditions

- As for the second growing season, plus
- Direct comparison in different locations
- Possible use of mixtures and coded samples in the applicant's premises
- Possible use of morphological distance
- Possible use of "supporting evidence" characteristics
- Contact with other DUS services

**Decision**- Acceptance, if one of these conditions is met; if clearly distinct based on i) Consistent differences at least among 2 out of 3 cycles, ii) Or a small differences + positive judgments of experts + supporting evidence characteristics. Other-wise Rejection.

**Essentially derived variety:** An application for registration of an EDV under sub-section (2) of Section 29 shall be submitted to the Registrar by or on behalf of the breeder & in the manner specified in Section 18 as if the word 'Variety' is substituted by the word 'EDV'. When the Authority is satisfied, it shall get examined such essentially derived variety to determine as to whether the essentially derived variety is a variety derived from the initial variety by conducting such tests and following such procedure as prescribed.

**Indian Reference Collection:** To start with, we may include the following in the Indian Reference Collection of given crop; i) All notified varieties which are in seed multiplication chain, i.e. whose breeder seed is being produced, ii) All important varieties in common knowledge, iii) All new candidate varieties of DUS test year and iv) Foreign varieties which are multiplied and certified in India.

**References**

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Protection of Plant Varieties and Farmers Rights" (PPV&FR) Rules 2003.
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# SEED PRODUCTION AND SUPPLY SYSTEM IN INDIA

**Agarwal D.K., Udaya Bhaskar K., Vijaykumar H.P., Sripathy K.V., Govind Pal, Ramesh K.V., Jeevan Kumar S.P., Boraiah K.M., and Bhojaraja Naik K.**
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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. 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.

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.

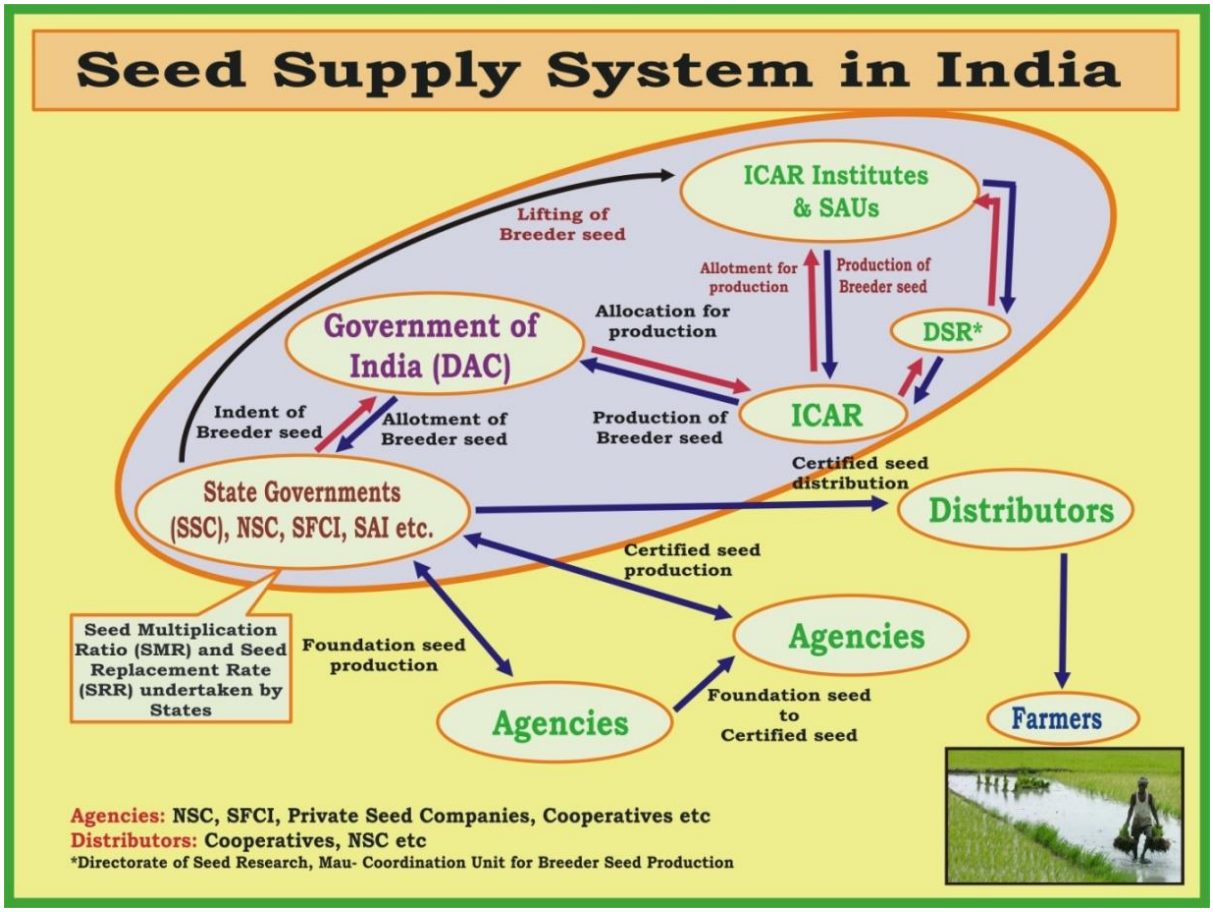
## Indian Seed Programme

As we all aware that India is one of the ancient agrarian societies. Nearly sixty percent of the country's population of over 114 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 US $ 4 billion (exhibiting CAGR of around 17 % during 2010-2017) and approximately 383 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 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.




*(Courtesy: DAC&FW, MoA&FW, New Delhi).*

**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- 1st 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)

**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**

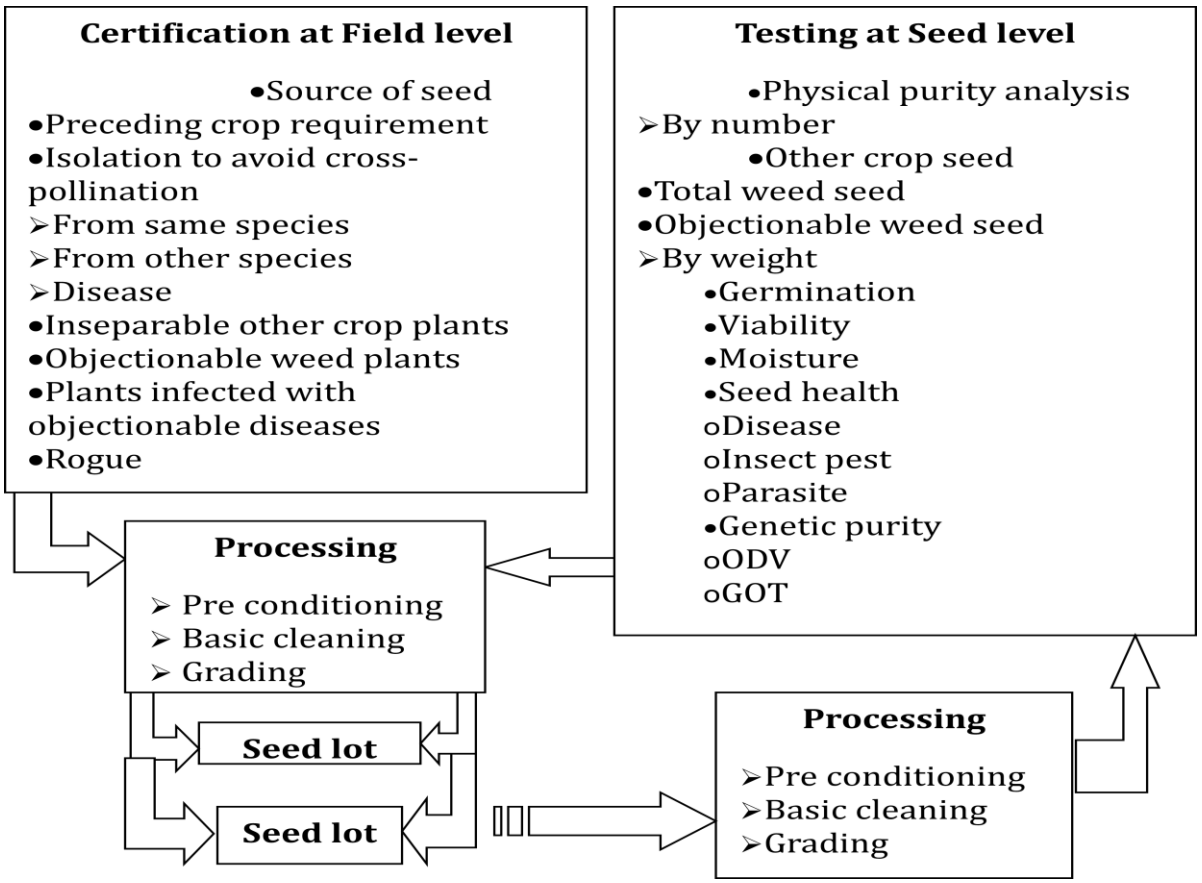
The Seed Rules are framed in the year 1968 giving wider scope for understanding various provisions of the Seeds Act 1966. The function of the Central Seed Testing Laboratory, Seed Certification Agency are elaborately dealt with. Labeling of any notified kind of variety of seed is made compulsory. It 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.
- Seed sampling procedures are dealt in detail.
- Maintenance of various records and issuing of memorandum in Form-VII by Seed Inspector to the dealer.

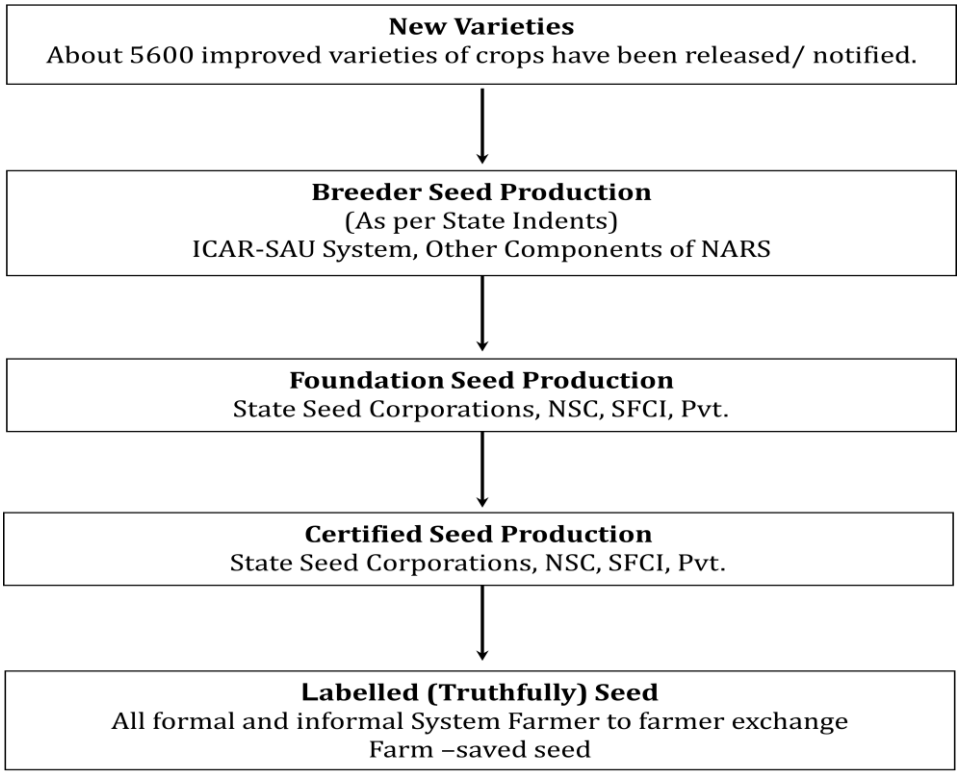
**Indian Minimum Seed Standards, 1971**

- These include standards both field level and seed level
- These standards were revised in 1988 and 2013 along with inclusion of more crops

**Steps of Seed Certification / Seed Quality Testing**



**Seed Production System in India**




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 343 lakh quintals in 2015-16. The projected seed requirement by 2020 AD is estimated at 360 lakh quintals by considering the proposed enhancement of seed replacement rates.

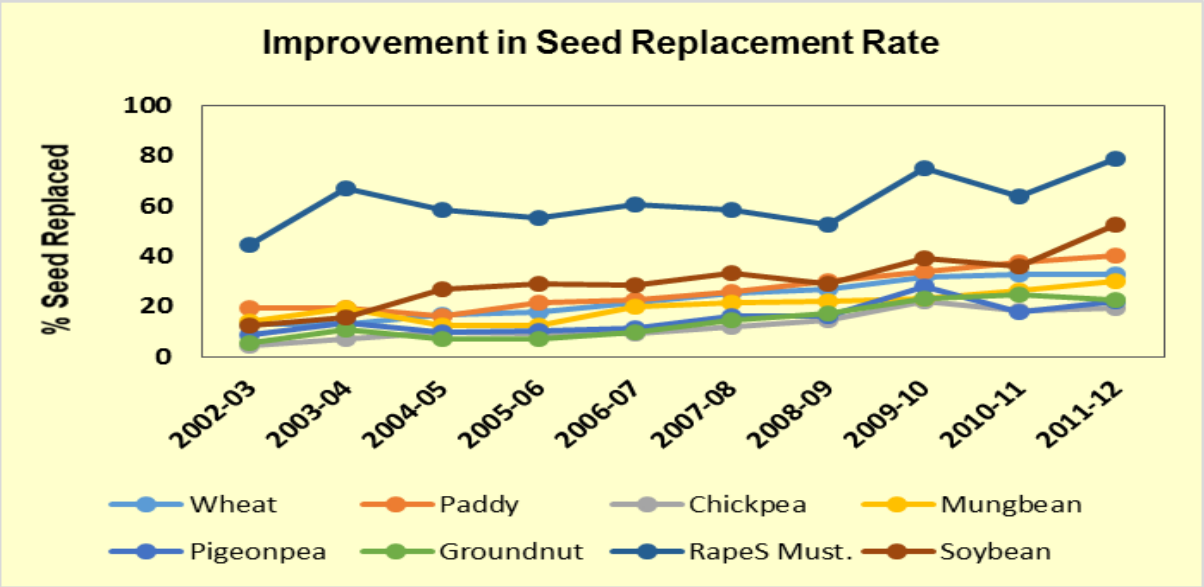
**Certified/Quality seed production/availability by public and private sector and their contributions (***Source: Agricultural Statistics at a glance 2016, MOA&FW, GOI.*)

| *Year* | *Quantity(million tonnes)* | | *Quantity of seed (million tonnes) produced by* | |
|---|---|---|---|---|
| | *Availability* | *Demand* | *Public sector* | *Private Sector* |
| *2007-08* | *1.94* | *1.80* | *1.12 (57.4%)* | *0.83 (42.6%)* |
| *2008-09* | *2.50* | *2.07* | *1.51 (60.2%)* | *1.00 (39.8%)* |
| *2009-10* | *2.80* | *2.49* | *1.71 (61.1%)* | *1.09 (38.9%)* |
| *2010-11* | *3.22* | *2.90* | *1.66 (51.6%)* | *1.56 (48.4%)* |
| 2011-12 | 3.54 | 3.30 | 1.81 (51.1%) | 1.73 (48.9%) |
| 2012-13 | 3.29 | 3.15 | 1.61 (49.1%) | 1.67 (50.9%) |
| 2013-14 | 3.47 | 3.35 | 1.68 (48.4%) | 1.79 (51.6%) |

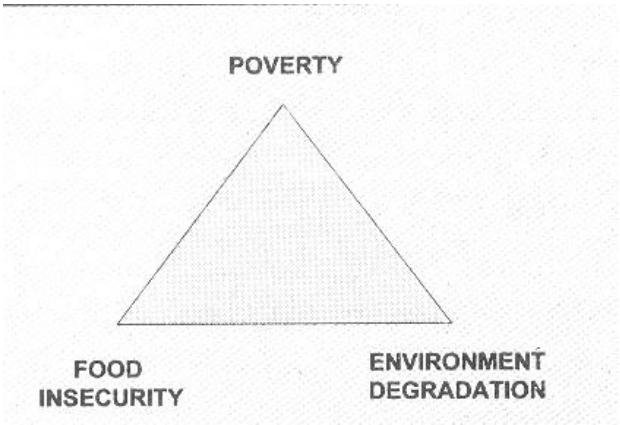
| | | | | |
|---|---|---|---|---|
| 2014-15 | 3.52 | 3.44 | 1.51 (41.2%) | 2.06 (58.8%) |
| 2015-16 | 3.43 | 3.37 | 1.47 (42.8%) | 1.96 (57.1%) |

**Seed Replacement Rate (SRR)**

Seed Replacement Rate (SRR) has increased from 25.2 to 32.6% (wheat); 25.9 to 40.4% (paddy); 44.2 to 56.6% (maize); 48.5 to 60.4% (pearl millet); 21.8 to 30.3% (moong / green gram); 23.9 to 34.4(urd / black-gram) and 14.3 to 22.5% (groundnut); 33.4 to 52.8% (soybean) in 2011 as compared to that of 2007.




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




**Future Needs and Projections:** A target of 252 million tonnes of food grains have been protected by 2015-16. 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.

# REGULATORY FRAMEWORK FOR SEED QUALITY ASSURANCE IN INDIA

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Quality seed of high yielding improved varieties is the foundation of agriculture. Other inputs in agriculture can contribute towards higher productivity only when good quality seed has been used. Seed is not only the carrier of the genetic potential of a variety, but it also is the carrier of other technologies. The production and distribution of high quality seeds was started systematically on the recommendation of 'Royal Commission on Agriculture in 1925. Later on with the introduction of high yielding and dwarf varieties of wheat and rice and hybrids in maize, sorghum and pearl millet in 1960's the seed production programme was further strengthened. Due cater the need of large quantity of quality seed 'National Seed Corporation (NSC) was established in 1963 and in due course of time other Seeds Corporation and Seed Certification Agencies were also established. In India we have two systems of seed supply viz., Formal and Informal. In case of informal system, the farmers are saving their own seed or they procure seed from the local farmers on payment basis or on barter system. Formal system comprises of public and private sector seed production agencies. As on now the public sector comprises of one national level corporations viz. National Seed Corporation (NSC), 19 State Seed Corporations (SSCs), 22 Seed Certification Agencies (SCAs), two Central Seed Testing and 122 State Seed Testing Laboratories (1 ISTA accredited and 20have ISTA membership) which is providing requisite strength in serving the seed industry and farmers. The research and development in the public sector is dependent on public research under the aegis of the ICAR institutes and SAUs. SAUs and ICAR Institutes are also engaged in breeder seed production and also in production of foundation and certified/truthfully labelled seed of their varieties. Besides, seed is also produced by the farmers under Farmers' Participatory Programme of several Institutes and under Seed Village Programme of the Government of India. Broadly 34 agricultural universities and 22 ICAR institutes across the country are engaged in seed production activities. State Agricultural Universities are taking up breeder seed production involving its KVKs to bring seed revolution in the country. On the other hand, private sector is highly fragmented, with more than 600 players (including domestic and multinational companies) and the top 10-12 seed producers account for more than two-third of domestic market.

To control the quality of seed various acts, regulations, orders have been implemented which were amended from time to time based on the requirements for quality assurance of the seed being produced in the country. Seed sector is governed by the Seed Legislations like The Seeds Act 1966 (Act No.54 of 1966) Passed on: [29th December, 1966], Come into force: October, 1969; Seeds Rules 1968; various amendments in the seed act and seed rules; Seeds Control Order,

1983 (under the Essential Commodities Act, 1955); New Policy on Seed Development 1988; Protection of Plant Varieties and Farmers' Rights Act 2001 (PPVFR Act); The Biological Diversity Act 2002; National Seed Policy 2002 and Seed Bill 2004 (under consideration) which helps in regulating the seed programme of the country to maintain the proper supply of quality seed and exchange of material. The various seed legislations for regulating the seed quality in India are discussed as under:

**I. Indian Seed Act 1966**

To control the quality of seed during production and marketing, Govt. of India enacted Seed Act in 1966 and framed the Seed Rules in 1968. The Seed Act was implemented in 1968. For effective implementation of the act, major provisions in the Act are:

- Establishment of Central Seed Committee
- Minimum Seed Certification Standards, 1971
- Certification voluntary but labeling is compulsory
- Label should contain necessary information
- Establishment of Central Seed Testing Laboratory
- Establishment of State Seed Testing Laboratories
- Establishment of Central Seed Certification Board
- Establishment of State Seed Certification Agencies
- Provision of Seed Inspectors
- Provision of Seed Analysts
- Law Enforcement

**Main features of Seed Act 1966**

1. ***Applicability***: Notification of kinds/varieties
2. ***Sanctioning Legislations***: Apex bodies like; Central Seed Committee, Central Seed Certification Board, State Seed Cert. Agencies, State Seed Testing Labs etc.
3. ***Regulatory Legislations***: Notification of kinds/varieties; regulations for seed sale; law enforcement machinery- seed inspectors, notification of CSTL, SSTLs, Seed Analysts, Appellate Authority etc.

**Central Seed Committee:** It is the main source of advice to Govt. on all matters related to seed, notification of kind/ varieties, seed standards, procedures for seed certification, seed testing labs, fees to be charged etc. Established in Ministry of Agriculture and its constitution is:

1. Chairman
2. Eight persons to be nominated by Central Govt. including at least 2 persons from representatives of seed producers/traders.

3. One person to be nominated from each of the states.
4. Term- 2 years

**Central Seed Certification Board:** Main function is to deal with all problems related to seed certification-established in Mini. of Agriculture. The constitution is:

1. Chairman
2. Four persons from states-Director of Agri.
3. Three members from SAUs- Director of Research
4. Thirteen persons to be nominated including four representative of seed producers/traders.
5. Term- 2 years

**State Seed Certification Agencies:** Main function of SSCA is to certify seed of notified kinds/ varieties, outline procedures for seed certification & maintain list breeders of seeds etc.

**Seed Certification:** International Crop Improvement Associations (1968) defined the purpose of seeds certification as "to maintain and make available to the farmers high quality seeds and propagating material of superior crop varieties, so grown and distributed as to ensure genetic identity and purity". In addition to genetic purity and identity, factors affecting physical purity such as fraction from obnoxious weeds and seed borne diseases, high germination, vigour and uniformity of seed etc. are also important. Seed Certification is a system that provides assurance to buyers of seed or a third party guarantee between the seed men and buyers**.**

**Phases of Seed Certification:** Certification shall be completed in six broad phases as given below:

i) Receipt and security of application.
ii) Verification of seed source, class and other requirements of the seed used for raising the seed crop.
iii) Field inspections to verify conformity to the prescribed field standards/factors which cause irreversible damage to the genetic purity or seed health .
iv) Supervision at post-harvest stage including processing and packing.
v) Seed sampling and analysis, including genetic purity test and or seed health test, if any, in order to verify conformity to the prescribed standards.
vi) Grant of certification and certification tags, tagging and sealing.

**Indian Minimum Seed Certification Standards 1971:** Since, it is not possible to maintain 100% purity hence, need for devising Minimum Field & Seed Standards without loosing seed quality/potential. Standards should be

"Achievable". Indian Minimum Seed Certification Standards were brought out in 1971. These standards were further revised in 1988 and 2013. These include standards both field level and seed level.

Two words are there which are important:

Minimum – for desirable parameters

Maximum- for un-desirable parameters

For details see website http://seednet.gov.in/

**Seed Law Enforcement:** The purpose of Seed Law Enforcement is:

1. To regulate the quality of seed sold to farmers.
2. With advance/modern agriculture, it becomes imperative to regulate the quality of seed through seed legislations & subsequent enforcement to ensure that the seed buyers are not at any disadvantage.
3. Notification of kinds or varieties.
4. Requirement for sale of seed.

**Regulatory Legislations**

1. Labelling with required information is compulsory.
2. Validity is defined.
3. No person can alter, obliterate or deface any mark or label.
4. Keeping record for 3 years.

**Regulation of seed sale of notified kinds or varieties- Seed Act 1966**

- No person shall, himself or by any other person on his behalf, carry on the business of selling, keeping for sale, offering to sell, bartering or otherwise supplying any seed of notified kind or variety, unless:
- Such seed is identifiable as to its kind or variety;
- Such seed conforms to the minimum limits of germination and purity specified under clause (a) of section 6;
- The container of such seed bears in the prescribed manner, the make or label containing the correct particulars thereof, specified under clause (b) of section 6; and
- He complies with such other requirement as may be prescribed.

**Seed Law Enforcement:** The purpose is to regulate the quality of seed sold to farmers. With advance/modern agriculture, it becomes imperative to regulate the quality of seed through seed legislations & subsequent enforcement to ensure that seed buyers are not at any disadvantage.

**Agency for Enforcing Regulations:** Seed Inspectors with prescribed qualifications shall be appointed by Govt. through notification. Govt. will also notify Seed Analysts & State Seed Testing Labs for testing of seed samples drawn by Seed Inspectors.

**Statutory Regulations for Seed Law Enforcement:**

i) Inspect as frequently as required- seed stores or sale points.
ii) Satisfy that conditions of certificates/ labels are intact.
iii) Procure & send samples of seed for analysis, which he has reason to suspect.
iv) Investigate complaints.
v) Maintain records.
vi) Detain containers, where ever required.
vii) Institute prosecution, where ever required.

**Procedure for Seed Law Enforcement:** Prescribed procedures are to be followed. Equipment & supplies like; identification card, copy of Seed Act/Rules, forms, other materials, cash for payment etc should be available and then packing/ sealing/ sending of samples should be done.
See details in books/Hand book

**II. Seeds (Control) Order 1983**

Inclusion of seeds as an essential commodity under the Essential Commodity Act 1955 brought the Seeds (Control) Order 1983.

- The Ministry of Civil Supplies declared seeds for sowing or planting (food crops, fruits, vegetables, fodder & jute) to be essential commodities under the Essential Commodity Act 1955.
- As per this order, Govt. has powers to control & regulate the production, supply & distribution of essential commodities.

As per this order, the GOI declared all the crop seeds whether notified or not as an essential commodity under the Seeds (Control) Order 1983 which requires:

1. Obtaining of seed licence to deal in seed sale/purchase, import/export & notify the authority for grant of licence.
2. Making seed available where ever required.
3. Notifying the Law Enforcing/Regulating Authorities.
4. Making compulsory issue of bills, display stocks & price list, maintenance of
   records & submission of returns to the Notified Authority.

**III. New Seed Policy 1988**

This Policy was formulated to provide Indian farmers with the access of best available seed & planting materials of domestic as well as imported quality. The Policy permits the import of seeds under OGL (Open General License), to make available to farmers high quality seeds of oilseeds, pulses, coarse grains, vegetables, flowers, ornamental plants, tubers, bulbs, cuttings & saplings of flowers with the objective to maximize production & productivity.

This Policy was formulated with special emphasis on:

- Import of high quality seeds & planting material.
- A time bound program to strengthen/ modernise plant quarantine facilities.
- Effective observance of procedures for quarantine/ post quarantine (PEQ).
- Incentive to encourage the domestic seed industry.

**IV. Protection of Plant Varieties and Farmers Right Act 2001**

AS per TRIPS Agreement Article 27 (3) b under WTO, the GOI has enacted the "Protection of Plant Varieties and Farmers Rights" (PPV&FR) Act, 2001, a '*sui generis system.* The Act is unique in a sense that it protects rights of not only breeders but farmers and scientists as well. It brings about a fine blend between the concepts underlying Convention on Biodiversity, International Treaty on Plant Genetic Resources for Food and Agriculture and TRIPS. It affords reasonable protection for development of new varieties without compromising the rights of farmers.

**The PPV&FR Authority:** The Authority shall be independent and permanent body vested with exclusive authority for implementation of the Act. It shall have a broad-based composition comprising scientists, state representatives, farmers/tribal, women's organization etc. The Authority shall consist of a Chairperson and fifteen members. Registrar General will be Member Secretary.

**General Functions of the Authority: (Section 8)**

1. Encouragement for the development of new varieties of plants and to protect the rights of the farmers and breeders.
2. In particular, and without prejudice to the generality of the foregoing provisions, the measures referred to in sub-section (1) may provide for:
3. The registration of new extant plant varieties subject to such terms and conditions and in the manner as may be prescribed.
4. Developing characterization and documentation of varieties registered under this act.
5. Documentation, indexing and cataloguing of farmers' varieties.
6. Compulsory cataloging facilities for all varieties of plants.
7. Ensuring that seeds of the varieties registered under this Act are available to the farmers and providing for compulsory licensing for such varieties wherever determined.
8. Collecting statistics with regard to plant varieties, including the contribution of any person at any time in the evolution or development of any plant variety, in India or in any other country, for compilation and publication.
9. Ensuring the maintenance of Register.

***Extent:*** All categories of plants except microorganisms.

**Period of Protection:** The total period of validity shall not exceed;
1. In the case of trees and vines, 18 years.
2. In other cases, 15 years from the date of registration of the variety.
3. In the case of extant varieties, 15 years from the date of the notification of that variety by the Central Govt. under section 5 of the Seeds Act, 1966 (54 of 1966).

**Other major provisions in the Act:**

- Breeders' Rights
- Researchers' Rights
- Farmers' Rights
- Rights of Communities
- Essentially Derived Variety
- Benefit sharing
- Extant variety
- Exclusion of certain varieties
- National Gene Fund
- Compulsory License
- Tribunal
- Report of Authority to be placed before Parliament
- Power of Central Government to give directions

**Breeders' Rights**

i) A certificate of registration for a variety issued under this Act shall confer an exclusive right on the breeder or his successor, his agent or license, to produce, sell, market, distribute, import or export the variety.
ii) A breeder may authorize any person to produce, sell, market or otherwise deal with the variety registered under this Act subject to such limitations and conditions as may be specified in the regulations.
iii) Breeder shall enjoy provisional protection of his variety against any abusive act committed by any third party during the period between filing of application for registration and decision taken by Authority [Section 24(5)]. Breeder should bring any act of such abuse to the notice of Registrar. Registrar has powers to issues directions in such cases (and also powers of a breeder – Sec. 24(5)

**Researcher's Rights (Section 30)**

i) The use of protected variety for conducting experiment or research.
ii) The use of a protected variety by any person as an initial source of variety for the purpose of creating other varieties.

iii) However, authorization of the breeder is required where repeated use of such variety as a parental line is necessary for commercial production of such other newly developed variety.

**Farmer's Rights**

- Right to save, use, exchange, share and sell farm produce of a protected variety, except sale of branded seed;
- Farmer's recognized as breeders, conservers. Preservers and cultivators of traditional varieties;
- Registration of extant varieties, including farmer's varieties.
- Farmer's to be compensated for lower than specifies yield;
- Farmer's exempted from payment of fees;
- Farmer's to be produce from acts of innocent infringement.

**Implications of PPV & FR Act**

- PVP will provide legislative framework and suitable environment for plant breeding and biotechnology research.
- PPV & FR Act will provide the favorable environment and needed support for enhanced availability of best planting material / seed to the farmers needed for enhancing food production in the country.
- The products developed would be protected and unethical practices during contract seed production can be prevented.
- The immediate effect of the act will be in terms of increased access to seeds developed by MNCs in other countries.
- Plant breeding activity by private sector will increase availability of improved diverse varieties and hybrids in crops like; maize, sunflower, cotton, tomato.
- However, clause pertaining to sale of protected varieties and benefits sharing in the Act may not induce private sector to increase research investment in plant breeding particularly in self-pollinated crops.

**V. National Seed Policy (2002)**

The Govt. of India formulated National Seed Policy-2002 to achieve enhanced seed replacement rate of various crops and there by food production targets to in the country. This would require a major increase in production of quality seeds, in which the private sector is expected to play a major role. It also intends to create facilitative climate for faster growth of localized seed industries, encourage import of useful germ plasm and boost export. It also aims to create a conducive atmosphere for application of emerging technologies like bio-technology for varietal development etc. and inflate R&D investments.

**Thrust Areas**

- Varietal development and Plant Variety Protection;

- Seed production;
- Quality assurance;
- Seed distribution and marketing;
- Infrastructure facilities;
- Transgenic plant varieties;
- Import of seeds and planting material;
- Export of seeds;
- Promotion of domestic seed industry; and
- Strengthening of monitoring system.

The National Seeds Policy will serve as a catalyst to meet the objectives of sustainable development of agriculture, food and nutritional security for the population and improved standards of living for farming communities.

**VI. Regulation of GM Crops**

For most transgenics, there are 3 kinds of bio-safety concerns:

- Bio-safety for human & animal health
- Bio-safety for environment
- Bio-safety for social & ethical concerns.

Prescribed tests for allergicity, toxicity, pollen flow & agronomic value have been followed. The safety of GM crops is established through various rigorous regulatory data generation work done in different parts of the world/ India. Some of the genes like Bt do not get activated in human gut due to various scientific reasons & hence question of any harm to human being does not arise.

Indian Acts, rules & regulations for handling of the GMOs have been framed under **Environmental Protection Act 1986 & Rules 1989**. Rules 1989 empowered a 3 Tier system:

**Institutional Bio-safety Committee (IBSC)** operates Research level approvals at Institute level.

**Review Committee on Genetic Manipulation (RCGM)** reviews all approved research projects/expts. The DBT, Mini. of Science & Tech. services RCGM for recognition

of IBSC & regulating research & bio-safety expts.

Finally, **GEAC (Genetic Engineering Approval Committee),** the Apex Committee under Ministry of Environment & Forest, GOI, regulates the GM crops. GEAC gives the required guidelines for testing.

**VII. Biological Diversity Act 2002**

- GOI will establish National Biodiversity Authority for implementation of the Act.

- Head office at Chennai.
- There is provision of State Biodiversity Boards/ Biodiversity Management Committees at local levels.
- No person shall without previous approval of NBA obtain any biological resource occurring in India or knowledge associated thereto for research or for commercial utilization or for bio-survey and bio-utilization.

**Main functions of BDA:**

- Make arrangement for proper conservation of our bio-diversity/ genetic resources.
- Documentation of all the bio-diversity/ genetic resources.
- Make available these bio-diversity/genetic resources or germ plasm to all stake holders for the benefit of seed industry or ultimately to the mankind.

## VIII. Export-Import Policy on Seed & Planting Material 2002-2007

- All import of seeds /PM would be regulated under the PQ Order 2003 or any amendment.
- Import license would be granted by DGFT/PPA recommended by DAC.
- All importers have to make available a small quantity of seeds to ICAR for testing/accession to the gene bank.
- The restrictions on export of all cultivated varieties seeds except; BS/FS of wild varieties; seeds of some selected crops like onion, berseem, cashew, rubber, pepper, niger etc have been removed w.e.f. 2004.
- Export of these seeds is allowed on case to case basis by DGFT
- Import by ICAR is allowed with out license following conditions specified by MOA.

## XI. New Seed Bill 2004/2010

To harmonize the Seed Act with the developments (combining Seeds Act, 1966, Seed Control Order, 1983, New Seed Policy, 1988, PPV&FR Act) & other rules/regulations, a need was felt to have a new Seed Act. The Government of India constituted a Seed Policy Review Group in 1998, which recommended a long-awaited shake-up and reform of the Indian seed laws. The new seed law is called as New Seed Bill 2004, which is being considered by the Parliament and need to be passed. It would replace the current 1966 Seeds Act.

**Major provision in New Seed Bill 2004**

- Seed certification will remain voluntary.
- There is a provision for Self certification.

- All varieties are to be registered in "National Register of Seeds" and are eligible for certification. Seeds of varieties or kinds not existing in "National Register of Seeds" shall not be permitted for marketing.
- Grant of registration will base on the information supplied by the breeder on "Agronomic performance" in multi location trials.
- The national level registration will be done by 'Registration sub committee' and at state level by 'State Seed Committee'.
- Transgenic varieties will be registered after getting the clearance under the provisions of the "Environment Protection Act 1986".
- Provisional registration for 2 years subject to clearance under Environment Protection Act 1986.
- Registration fee structure would be same as applicable to other seed (non-transgenic).

**Comparison of Seeds Bill 2004 and Seeds Act 1966**

| | **Seeds Bill 2004** | **Seeds act 1966** |
|---|---|---|
| Definition | "Agriculture" includes horticulture, forestry, cultivation of plantation, medicinal and aromatic plants | "Agriculture" includes horticulture |
| Registration | All seeds for sale must be registered | Only varieties notified by the government need to be registered |
| Seed committee | Constitutes Central and State Seed Committees<br>A Registration Sub-Committee would register seeds of all varieties | Constitutes Central Seed Committee. The central government, after consulting with the CSC, may notify a seed in order to regulate the quality of seed |
| Transgenic varieties | Special provisions for registration of transgenic varieties of seeds | No provision for transgenic varieties of seeds |
| Compensation to farmers | Provides for compensation to farmers under the Consumer Protection Act, 1986 in the event of under performance of seeds | No specific provision for compensation mentioned in the Act |
| Role of seed inspector | The Seed Inspector has the power to enter and search as well as | Under CrPC warrant signed by DM, SDM or first class magistrate is necessary |

| | | |
|---|---|---|
| | break open container or break open doors, without a warrant | |
| Registration of nursery | Every horticultural nursery has to be registered with the state government and has to maintain records of layout plan, source of every planting material etc | No specific provision |
| Certification | Seed producers would be permitted to self certify their seed under certain conditions | Certification is voluntary |

**Provisions for farmers**

"Nothing in this Act shall restrict the right of the farmer to save, use, exchange, share or sell his farm seeds and planting material, except that he shall not sell such seed or planting material under a brand name or which does not conform to the minimum prescribed limit of germination, physical purity, genetic purity".

# PRINCIPLES AND PRACTICES OF SEED PRODUCTION IN SELF POLLINATED CROPS

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Seed, in broad sense, is the propagating material; fertilized matured ovule together covered with seed coat, tuber, bulbs, rhizome, roots, cuttings, setts, slips, all types of grafts of agriculture, sericulture, silviculture and horticultural plants used for sowing or planting and raising healthy seedlings. Thus, seed is the most vital and crucial input for crop production, one of the ways to increase the productivity without adding appreciably to the extent of land now under cultivation by planting quality seed. The importance of quality seeds has been recognized from the time immemorial. Good quality seed is essential for the production of a crop suitable for export. Although the importance of seed was recognized in ancient agriculture, the need for organized seed production was identified only at the beginning of 20th century. The systems have been designed to provide quality control during seed production.

Production of quality seed is most important and specialized technology on Agriculture based economies. It is followed up after the variety improvement programme in crops. Hence, the seed producing agencies (Public & Private) have an obligation to maintain quality seed production programme prior to crop season with required quantity of seed. It is seed production chain: Normally the crop improvement programme takes a decade to develop a variety. Its multiplication starts when a variety is identified for its higher performance in advance trials. The originality of a variety will deteriorate if maintenance breeding/seed production chain is not monitored during the various growth stages of a crop. The deterioration of the variety could be due to (i.) Natural out cross, (ii.) Mechanical mixture during harvest/hulling (iii.) Developmental variation, (iv.) Residual heterozygosity (v.) Mutations (vi.) Selective influence of disease.

Certain principles needed to be followed in quality seed production of crops are i.e. maintained high genetic purity, germination percentage, viability and vigour. The seeds should be free from weeds, seed born disease and pests. Pollination is the mechanism by which pollen (male gamete) of the parent plant reaches to egg cell (female gamete) of the flower. Pollination mechanism divides crop in two categories a.) Self pollinated crops viz. rice, pulses etc. b.) Cross Pollinated Crops viz. Maize, pearl millet, brassica etc. The seed propagated crops are classified into two categories based on pollination made (i) Self pollinated (ii) Cross pollinated crops. The chances of out cross in self-pollinated crops are less than 5% while the cross pollinated crops may have 100% out cross pollination. The cross pollinating agents could be Abiotic- wind or water (anemophily and hydrophily). In Biotic- living agent's viz. bees, insects, rats,

butterfly, birds and also humans (manipulation of crosses) are involved in cross pollination. In present write up the Seed Production in Self Pollinated Crops has been briefly discussed.

**Maintenance of genetic purity**

Maintenance of genetic purity of self pollinated crop varieties during the course of seed production and multiplication is most important condition. Self-pollinated crops are 100% homozygous for its genetic constitution and no loss of plant vigour is envisaged in self-pollinated crops. It is therefore, important to maintain its homozygosity throughout its multiplication in seed generation system. Therefore, we need to know the various causes for deterioration of genetic purity of varieties.

The genetic purity of a variety or trueness to its type deteriorates due to several factors during the production cycles. Kadam (1942) listed the seven important factors responsible for deterioration of varieties.

**1. Developmental Variations:** When seed crops are grown under environments with differing soil, fertility, climate photoperiods, or at different elevations for several consecutive generation's developmental variations may set in as differential growth responses. It is therefore, preferred to grow the varieties of crops in the areas of their natural adaptation to minimize developmental shifts.

**2. Mechanical Mixtures:** Mechanical mixtures, the most important reason for varietal deterioration, often take place at the time of sowing if more than one variety is sown with the same seed drill, through volunteer plants of the same crop in the seed field, or through different varieties grown in adjacent fields. Two varieties growing next to each other field is usually mixed during harvesting and threshing operations. The threshing equipment is often contaminated with seeds of other varieties. Similarly, the gunny bags, seed bins and elevators are also often contaminate, adding to the mechanical mixtures of varieties. Roguing the seed fields critically and using utmost care during seed production and processing are necessary to avoid such mechanical contamination.

**3. Mutations:** Mutations do not seriously deteriorate varieties. It is often difficult to identify or detect minor mutations occurring naturally. Mutants such as 'fatuoids' in oats or 'rabbit ear' in peas may be removed by roguing from seed plots to purify the seeds.

**4. Natural Crossing:** Natural crossing can be an important source of varietal deterioration in sexually propagated crops. The extent of contamination depends upon the magnitude of natural cross-fertilization. The deterioration sets in due to natural crossing with undesirable types, diseased plants, or off types. In self-fertilized crops, natural crossing is not a serious source of contamination unless variety is male sterile and is grown in close proximity with other varieties. The natural crossing, however, can be major source of contamination due to natural crossing are the breeding system of the species,

isolation distance, varietal mass and pollinating agent. The isolation of seed crops is the most important factor in avoiding contamination of the cross-fertilized crops. The direction of prevailing winds, the number of insects present and their activity, and mass of varieties are also important considerations are contamination by natural crossing.

**5. Minor Genetic Variations:** Minor genetic variations can occur even in varieties appearing phenotypically uniform and homogenous when released. The variations may lost during later production cycles owing to selective elimination by the nature. The yield trials of lines propagated from plants of breeder's seed to maintain the purity of self-pollinated crop varieties can overcome these minor variations. Due care during the maintenance of nucleus and breeder's seed of cross-fertilized varieties of crop is necessary.

**6. Selected Influence of Pest and Diseases:** New crop varieties often are susceptible to newer races of pests and diseases caused by obligate parasites and thus selectively influence deterioration. The vegetatively propagated stock also can deteriorate quickly if infected by virus, fungi or bacteria. Seed production under strict disease free conditions is therefore essential.

**7. The Techniques of the Plant Breeder:** Serious instabilities may occur in varieties owing to cytogenetic irregularities in the form of improper assessments in the release of new varieties. Premature release of varieties, still segregating for resistance and susceptibility to diseases or other factors can cause significant deterioration of varieties. This failure can be attributed to the variety testing programme. In addition to these factors, other heritable variations due to recombination's and polyploidization may also take place in varieties during seed production, which can be avoided by periodical selection during maintenance of the seed stock.

**Seed Production in Self Pollinated Crops**

Mere development of a variety is not enough. Benefits from an improved variety can be realized only if good quality seed is made available to farmers in sufficient quantities. To ensure availability of good quality seed in sufficient quantities a systematic seed production programme is very essential. In the generation system of seed multiplication being followed in India there are three recognized classes of seed i.e. breeder seed, foundation seed and certified seed. Certified seed is actually sold to the farmers to raise the commercial crop. Continuous availability of high quality Nucleus Seed, which is the result of Maintenance Breeding, is required to sustain this seed multiplication chain.

**Nucleus Seed Production *(Maintenance Breeding)***

Maintenance of high varietal purity is a never ending task. Off-types in a variety could arise due to the reasons; Segregation of residual heterozygosity (Multiple crosses), Spontaneous mutations, Natural out-crossing and Admixtures

Proper maintenance of a variety is important to ensure that it retains its yield

potential and other desirable attributes. Maintenance Breeding serves the following purposes:

- It helps in purification and maintenance of a variety and consequent production of nucleus seed.
- The use of nucleus seed in turn reduces the amount of rogueing required in large breeder seed production plots. In fact some offtypes can be detected only at nucleus seed production stage.
- It helps in extending the productive life of a variety.
- It will help in meeting the uniformity criteria in DUS testing, provided the maintenance breeding is started at an early stage.

Normally the breeder starts nucleus seed production of a variety only after the variety gets identified for release. It is also a fact that many promising lines in co-ordinated trials are not considered for promotion due to large number of off-types. Now for getting a new variety protected under the PPV&FR Act the variety has to pass the DUS test. It is suggested that the breeder should start maintenance breeding procedure at least 2 seasons before he expects the material to enter DUS testing and NIVT (National Initial Varietal Trial). This will ensure supply of genetically pure seed for above tests. This would be the starting point of Maintenance Breeding and should be continued once a variety has been identified and released for general cultivation.

**Procedure of Nucleus Seed Production**

Nucleus seed is produced by growing ear rows in wheat, panicle rows in rice, cluster rows in cowpea and plant rows in mungbean, chickpea etc. The various steps to be followed for production of nucleus seed are as given below:

1. Select between 400-500 typical ears/panicles/clusters/plants at maturity stage from the seed multiplication plot of a given genotype/variety.
2. Harvest and thresh them separately. Examine the seeds for colour, shape and size and reject the seeds of those ears/panicles etc. which are not representative of the variety.
3. During the next cropping season from the seeds of each selected ear/panicle etc. grow a row of suitable length. This is known as an Ear/Panicle/Plant-Row. Grow between 300-400 such rows per variety.
4. Examine these rows periodically throughout the growing season particularly at early growth stage, ear/panicle emergence, early dough stage and at maturity. Reject rows that show off-types (s). Such rows should be rejected as and when detected.
5. The off-types can be w.r.t. to any character such foliage colour, auricle pigmentation, days to flowering, waxy bloom, plant height, ear/panicle colour, shape and density, various glume characters etc. Diseased as well as agronomically poor looking rows should also be rejected.

6. When an off-type is detected after flowering stage, then the rows on either side of the row showing off-type should also be rejected to minimize contamination from offtype plants.
7. Rows which are true to type of the original variety for all externally observable characters are selected. From these true to type rows between 400-500 ears/panicles/clusters/plants are selected for the next cycle of nucleus seed production.
8. Harvest and thresh the selected rows separately. Examine the seeds for colour, shape and size and reject the seeds of those rows which show deviation. The bulked seed of the remaining true to type rows is the Nucleus Seed.

In some crops like wheat where seed rate is high the nucleus seed produced above (around 100kg) may not be sufficient in some cases when the breeder seed indent is more than 30-35 quintals. In such cases the nucleus seed produced by growing ear rows is referred to as the Nucleus Seed Stage-1 (NSS-1). The seed of true to type ear rows in stage-1 is kept separate and used to grow Ear-Row Progeny Plots in the next season. The size of ear row progeny plots can vary depending upon the nucleus seed requirement. This provides another opportunity to reject some of the plots which escaped detection during Stage-1. The seed from uniform and true to type ear row progeny plots is bulked to produce Nucleus Seed Stage-2 (NSS-2).

**Method of Planting (Wheat as an Example)**

Although wheat is a self pollinated crop but it has been observed over the years at IARI-Regional Station, Karnal that some outcrossing does occur even after following the recommended isolation distance of 3 meters between varieties. Some varieties like HD 2428 are more prone to outcrossing than others. Subsequent isolation distance studies in wheat revealed that outcrossing did occur even up to 5 meters and main contamination was in the first 3 to 4 border rows. Therefore, 3 meters isolation may be appropriate for large seed production plots as the contamination gets diluted due to the large size of the seed lot. However, 3 meters isolation may not be appropriate for maintenance breeding. Therefore, to ensure production of nucleus seed of highest genetic purity the ear-rows/ear-row progeny plots of a variety should be planted in such a way that these are surrounded by seed crop of the same variety. Even if there will be any outcrossing it will be with the same variety. This method has another advantage. The seed crop acts as a reference standard in case of any doubt regarding the off-type. Despite the fact that this improved method is inconvenient in terms of management and in recording observations, this ensures the production of genetically pure nucleus seed.

At locations where isolated plots are not available, nucleus seed production plots of different varieties can be separated by growing a strip of relatively tall non-lodging crop which is not cross-compatible with the main crop. Such tall

crops act as physical barrier and prevent outcrossing between different plots.

**Breeder Seed Production**

Seed is the cheapest and most effective agricultural input. By being often the central input in the production input package, quality seeds of improved varieties play an important role in modern agriculture. Raising of a seed crop differs from raising of a commercial crop in several respects. Special methods and precautions are needed to produce seed of desired quality. Important considerations in the production of high quality seed are discussed below:

1. The breeder's stock seed from the nucleus is sown on clean, fertile land, having grown no crop of the same kind during the previous year.
2. The area required for planting breeder's seed stock is about 1.2 ha for wheat and 3 ha in the case of transplanted rice.
3. The field should be properly isolated.
4. Adopting standard agronomic practices of sowing, intercultural and harvesting, the seed should be produced at the experiment station in the area where the new variety has been bred.
5. Row spacing should be sufficient to permit examination of plants in rows for possible mixtures or off types.
6. Plants not typical of the variety should be pulled and removed (roguing) before flowering as was done for nucleus / breeder's seed stock.
7. Where plants are removed after flowering and pollen has escaped all surroundings plants within one meter should be discarded.
8. The breeder's stock is harvested, threshed with clean equipment, and bagged and labelled.
9. The seed should be about 100 % pure to its variety. This breeder's seed is now ready for multiplication as foundation seed.
10. The breeder's stock should be continued each year to furnish a fresh stock of seeds to the growers of foundation stock until the variety is replaced by newer ones.
11. The genetic purity of the breeder's seed of the established varieties of crops can be maintained by growing the crop in isolation and by rigorous roguing during different phases of crop growth.
12. The purity can be further enhanced by bulk selection, where in 2000-2500 plants typical of the variety are selected, harvested and threshed separately.
13. The seeds are critically examined, discarding off types, if any. The uniform seeds are bulked to constitute breeder's seed.
14. This process may be continued until deterioration sets in, changing plant characteristics of economic importance. Breeder's seed, therefore, needs to be included in yield tests.
15. The breeder must carry over at least enough seed to safeguard against the loss of variety due to complete failure of crop during the multiplication of foundation seed.

16. The production of breeder's seed is an expensive process, with the associated risks of contamination by repeated multiplication and loss due to adverse growing conditions. Such risks of contamination by repeated multiplication and loss due to adverse growing conditions. Such risks can be minimized and the continuity of seed programme better assured by producing sufficient breeder's seed at one time to meet the requirements of two the three productions of foundation seed (carryover of breeder's seed)
17. The carryover seed must be stored under optimum conditions in order to maintain its vigor and viability.

**Certified Seed Production (Foundation-Certified)**

In the Indian generation system of seed multiplication, there are three recognized classes of seed i.e. breeder seed followed by foundation seed and certified seed. Certified seed is actually sold to the farmers to raise the commercial crop. There is another category of seed known as Labeled Seed in which case there is no certification but labeling is compulsory. Tag colour and size have also been specified for each class/category of seed.

| Class of Seed | Tag Colour | Tag Size |
|---|---|---|
| Breeder | Golden Yellow | 12 cm × 6 cm |
| Foundation | Both side White | 15 cm × 7.5 cm |
| Certified | Both side Azure Blue | 15 cm × 7.5 cm |
| Labelled Seed | Opel Green | 15 cm × 10 cm |

If the tag is to be fixed on a smaller container then the size of the tag can be reduced proportionately (except for breeder seed). However, length and breadth ratio and contents would remain the same.

**Seed Certification Standards**

In India seed certification standards have been prescribed for foundation and certified seed. There are two types of standards; General Standards which are basic and applicable to all the crops and Specific Standards which relate to individual crops. General standards include classes and sources of seed, certification procedure, seed standards of genetic purity and for insect damage, grow-out test, validity period of certificate etc. Specific standards have further two categories i.e. field standards, which apply to standing crop and seed standards which are applicable at seed level. Field standards include isolation requirement, maximum permissible level of off-types, inseparable other crop plants, objectionable weed plants, pollen shedders (in male-sterile or A lines), plants infected by seed borne diseases etc. Seed standards relate to genetic purity, physical purity, germination, other crop seeds, weed seeds, moisture content etc.

It is a fact that such standards have not been prescribed for breeder seed.

Desirably the standards for breeder seed should be stricter than prescribed for foundation seed. However, in practice, foundation seed standards are also followed for production of breeder seed.

**Agro-climate**

1. The crop variety to be grown for seed production must have a suitable Agro-climate, adapted to the photoperiodic and temperature conditions prevailing in that location.
2. Specific selected locations would be needed to economically grow crop varieties sensitive to photoperiodism (short days viz., long days) and temperatures.
3. The regions with moderate rainfall, humidity and extreme temperatures.
4. Most agronomic crops require a dry sunny period and moderate temperatures for flowering and pollination.
5. Excessive dew and rains affect normal pollination , resulting in poor seed set.
6. Extreme temperatures may cause desiccation of pollen and poor seed set.
7. Very hot and dry weather conditions adversely affect the flowering of several crops, especially vegetables, legumes and fruit crops, which fail to set seed. These crops invariably require cooler climates with low atmospheric humidity to flower and pollinate normally.
8. Oil seed crops may tolerate hot weather during flowering, but very high temperatures can result in premature flowering and the production of poor quality seeds.
9. Extreme cold temperatures also damage seed quality in the early phases of seed maturation. Thus, locations with extreme agroclimate (summer hot and cold winters) are generally not suitable for seed production.
10. Excessive rainfall conditions normally result in a higher incidence of pest and diseases making the harvesting and other operations of seed production extremely difficult. They may also cause delayed maturity and pregermination of seed in many standing crops.
11. A mature seed crop becomes increasingly susceptible to shattering, strong winds, and heavy rainfall.
12. Ample sunshine, moderate rainfall, climate and absence of strong winds are ideal for the production of high quality seed.

**Location**

Agarwal (1980) lists the following desirable characteristics of land selected for seed crops:

1. The seed plot should have soil texture (light, well drained) and fertility characteristics as required by the crop.
2. The plot should be free from volunteer plants and seeds of weeds and other crop plants.
3. The soil of the selected plot should be comparatively free from soil borne

diseases and pests.
4. The same crop or variety thereof should not have been cultivated during the previous season on the plot selected for seed production.
5. The plot must be levelled and feasible for isolation as per the requirement of certification standards.

**Land Selection**

Land to be used for seed production should be fertile, well drained and free of volunteer plants (self sown plants). Volunteer plants is a problem in legumes and rice. However, when rice is transplanted after puddling the soil, volunteer plants are rare. In case of groundnut the seed crop should not be planted on land on which another variety was planted in the previous two seasons. There is no problem of volunteer plants in crops like wheat. Fields heavily infested with objectionable weeds should be avoided unless effective weed control measures are available.

**Isolation**

The seed crop must be sufficiently isolated from nearby fields of the same or other contaminating crops as per the requirements of certification standards. In strictly self-pollinated crops it is mainly to avoid mechanical mixture from adjoining plots. Isolation requirement varies from a few meters (3 m for wheat, paddy etc., 10 m for green gram, black gram, cowpea, chickpea, lentil, field pea etc. and 50 m for linseed for foundation seed production) to 200 meters for foundation seed production of parental lines of paddy hybrids.

Sources of contamination could be other varieties of the same crop, same variety not conforming to varietal purity requirements of the category of seed under production. Some time isolation is also required from sources of seed borne diseases (in wheat isolation requirement from other varieties is only 3 m whereas isolation from fields of wheat, triticale and rye with infection of loose smut disease in excess of 0.10% and 0.50% is 150 m in case of foundation and certified seed, respectively).

Genetic contamination with unwanted pollen can be minimized by keeping in mind prevalent wind direction at flowering stage at a particular location. Fields to be selected for seed production should be chosen in such a way that they do not lie in the downwind direction side of the possible contaminant source.

On a small scale in nucleus / breeder's seed production, isolation may be achieved by enclosing individual flowers or by removing male flower parts and employing artificial pollination. Even after the seed crop is harvested, effective isolation of seed from different varieties is essential to avoid mechanical contamination. Bags and other equipment must be thoroughly clean to maintain seed purity.

**Selection of Variety**

1. After the land is prepared for improving germination, including freedom from weeds and uniform irrigation, the selected crop variety is carefully planted.
2. The variety selected should suit the prevailing agroclimatic conditions, high yielding and possessing desirable attributes such as disease resistance, earliness and grain quality. Similarly the seed should be known purity, appropriate class, and obtained from an authorized official agency.
3. The seed may require treatment before sowing, if not treated already. Seed treatment may be given with appropriate fungicides or involve bacterial inoculation for legumes or for breaking dormancy.
4. Seeds having hard seed coats may require soaking in water overnight to facilitate germination.
5. The seed must be planted at its normal planting time in soil having adequate moisture content for germination. Lower than usual seed rates of commercial crops will facilitate the roguing and inspection of seed crops.

**Sowing Method**

The seed crop should be sown in lines with seed drill/planting equipment. One direction line sowing helps in cultural operations and inspection. Before and after sowing one variety, the seed drill needs a thorough cleaning of the pipes, seed cups and box. All cracks and crevices in the seed box as well as around and under the collecting and distributing mechanism must be cleaned with a stiff wire.

To ensure that physical mixture due to negligence at sowing time does not occur, it is desirable that phenotypically contrasting varieties are alternated. In such a situation any seed drill resultant rogues can be easily spotted in the field and rogued out.

The seed crop must be sown in such a may that it facilitates the movement of the personnel to effectively execute rogueing. Skipping one row after every eight rows by plugging the central seed dropper pipe of a 9-tyne seed drill has been found to be very convenient and effective in case of wheat at IARI-Regional Station, Karnal. Similarly, paired row planting in rice, green gram and chickpea is very useful.

Inter-cropping is permitted for production of certified seed class of only oilseeds and pulses. Foundation seed class should be raised strictly as a single crop only. The crops selected for inter-cropping should belong to different genus and preferably with different maturity. Other cropping patterns like mixed cropping is not permitted.

In hybrid seed production male and female parents must be planted in the recommended row ratio along with border rows of the male parent. Also row direction is very important in wind pollinated crops and it should be kept nearly perpendicular to the prevalent wind direction at flowering time.

**Agronomic Practices**

Cultural practices such as seed bed preparation, fertilization, weeding, irrigation etc. are usually the same for seed crop as recommended for commercial crop. All recommended agronomic practices should be followed to provide conditions for optimal growth and development of plant and seeds which favour production of healthy and vigorous seed. Clean cultivation with proper weed control during seed production makes subsequent cleaning and grading easier. Phosphate and potassium are generally more important for seed crops than for commercial crops and recommended doses must be applied. However, slightly less than the recommended amount of nitrogen should be used in the seed crop especially of cereals to minimize lodging. A crop that lodges badly cannot be effectively rogued and inspected and will not be approved.

***Irrigation:***

1. Because drier climates are more suitable for producing high quality disease free
seeds, irrigation is essential to obtain good seed yields.
2. Irrigation may be required before planting and at suitable intervals up to flowering.
3. One or two irrigation's may be desirable for many seed crops.
4. The frequency of irrigation and amount of water supplied depend upon the physical texture of the soil and crop requirements.
5. Maximum benefits from irrigation's can be derived only with adequate crop nutrition in the form of organic manure and fertilization, especially readily available sources of nitrogen and phosphorus.
6. Seed crop is rather sensitive to moisture stress at the vegetative, flowering and maturity stages. Adequate soil moisture is also necessary for uniform seed germination necessary to further crops stand and good seed yields.
7. Both excessive moisture conditions and prolonged drought will adversely affect germination, growth and development of the seed crop.
8. Water may be applied by surface irrigation, sprinkler, drip or overhead irrigation or subsurface irrigation.
9. The irrigation should be stopped 2-3 weeks before seed maturity to ensure the dried conditions needed for harvesting.

***Plant Nutrition:***

1. Adequate amounts of nitrogen, phosphorus, potassium and other essential minerals are crucial for the proper growth and development of the seed crop. It is, therefore necessary to know the nutritional requirements of any individual seed crop and to ensure proper nutrition at all the stages of crop growth.
2. Split applications of nitrogen are generally advocated to avoid lodging of a crop due to excessive vegetative growth.
3. Application of nitrogen at the time of flowering leads to an increase in yield and quality of the seed of most crops.
4. In some early crops nitrogen dressing at flowering may tend to delay

ripening.
5. While most grasses and peas are benefited by early applications of nitrogen, lettuce crops respond well to nitrogen application at the time of flowering.
6. Phosphorus and potassium favour root growth, increased strength of straw fruiting and seed development. They also hasten plant maturity and increase disease resistance.
7. Potassium improves the photosynthetic efficiency of plants and favours both protein and lipid metabolism in oil seeds.
8. Deficiencies of other essential secondary and micronutrients also need to be monitored carefully using soil test measures.

***Plant Protection:***

1. Effective control of all pests, including diseases and insects, is essential to produce a healthy seed crop. In addition to heavy reductions in seed yields, diseases and pests damage the quality of the produce.
2. Planting seed chemically treated with the appropriate fungicides effectively checks the seedling and many of seed borne diseases.
3. Applying the appropriate fungicides and insecticides in proper quantities and at the right time can effectively control most seed crop pests.
4. Adoption of appropriate schedules of plant protection and roguing of diseased plants and ear heads from time to time will further check the spread of disease and insects.

***Weeding:***

Production of high quality seed requires through control of weeds on the seed plot. In addition to reduction in seed yield, weeds are often a source of contamination by way of mixing at the time of harvest. Weeds in the seed plot or nearby areas may also harbor a number of pests and diseases. Effective control of weeds at all the phases of crop growth is essential and they must not be allowed to flower or set seed in any case. Planting seed crops on clean, fallow land or following crop rotations is generally recommended to keep at a minimum. Hand weeding, intercultural operations or chemical weed control may be necessary.

**Pollination:**

Supplementary pollination provided by honey bees in hives in close proximity to seed crops that are cross pollinated by insects may be necessary to ensure good seed set and thereby increase seed yield.

**Roguing:**

Roguing is the removal of off-type plants and is an important aspect of seed production to maintain varietal purity. Adequate and timely roguing constitutes the single most important operation in seed production. Rogues differing from normal (weak or sickly plants, bolters and off types) are pulled out and discarded at the earliest possible phases, before flowering, especially in cross

pollinated crops to avoid genetic contamination. Any plant which does not conform to the characteristics of the variety is called an off-type. Off-types are generally considered to arise from segregation of residual heterozygosity, spontaneous mutations, natural out-crossing with other varieties or admixtures. Off-types could be w.r.t. any character such as plant height, days to flowering, waxiness, pigmentation, ear shape, ear size, ear density, ear colour etc. It is essential that off-types are removed before they flower to avoid contamination from off-type plants.

In addition to roguing all plants that do not conform to the variety description, inseparable other crop plants, objectionable weeds as well as plants infected with seed-borne diseases should also be removed. As a general rule, the off-types should be removed and taken away from the seed production plot and destroyed. Light levels are important and dull, excessively bright and windy days should be avoided. The back of the person doing rogueing should be towards the sun. This facilitates easier detection of off-types.

**Field Inspection:**

The production of foundation and certified seed is supervised and approved by State Seed Certification Agencies. The seed production plots are inspected by the certification staff. The number of inspections varies from a minimum of two to four. Those plots which conform to field standards of certification are approved. Breeder seed has been kept out of the purview of certification as it is not meant for public sale. Moreover, its production is under the direct supervision of a qualified plant breeder. However, breeder seed crop is monitored by a joint inspection team of plant breeders and officials of State Seed Certification Agency and National Seeds Corporation.

**Harvesting:**

1. After completion of essential cultural operations and approval of seed fields for certification, the crop is ready for harvest. The appropriate time of harvest to ensure maximum seed yield and quality is of great significance.
2. Fully matured seed is easily harvested and cleaned with minimal harvest losses. While early harvests may make combining difficult, with increased losses in threshing and cleaning, harvesting at later stages may result in increased losses due to weather, lodging, seed shattering and pest and disease.
3. Seed moisture content is a good indication of the optimum time of harvest. Combines do not normally operate well above 15% seed moisture. While soybeans may be harvested best at a seed moisture content of 13% for wheat the best moisture content varies from 15 to 17%. Harvesting of seed crops at seed moisture contents of less than 20% minimizes mechanical damage to seed.
4. A seed crop may be harvested manually or mechanically, taking care to avoid mechanical injury to seeds during harvesting and threshing operations. Care must also be taken to avoid any chance of mechanical mixing of seeds and

maintain lot identity.
5. The germination of seed is of vital importance and the threshers, combine harvesters etc. must be properly set up to thresh the seed without inflicting damage to the seed.

**Drying:**
1. Cemented threshing floors or use of tarpaulins is preferred to maintain the quality of seeds. A crop may be harvested by directly combining in the field using mechanical combines. Sun drying of seeds on threshing floors, spreading the seeds in thin layers, may be necessary to reduce its moisture content and improve the storage quality.
2. Drying of a seed crop to its safe moisture limit to preserve its viability and vigour must be carried out rather quickly. If the seed is to be dehydrated mechanically, it should be taken to the processing plant soon after harvesting.
3. Care must be taken at all stages to avoid mechanical mixing and to minimum the identity of seed lots.

**Post-harvest Handling:**
All our efforts in roguing and care during earlier stages may go waste if proper care is not taken during harvesting, threshing, processing, seed treatment, packaging etc. to avoid mechanical mixing. Correct labelling is very important. The threshers, combine harvesters, trailers, threshing floors, processing machinery etc. should be thoroughly cleaned in between handling of different varieties.

**Seed Testing, Labelling and Storage:**
After processing of a given lot, the representative sample is sent to notified seed testing laboratory for analysis. Seed testing is done as per ISTA Rules. If the seed test report is satisfactory i.e. the seed meets the prescribed seed standards then a given seed lot is approved and tags and certificates are issued by the certification agency to the seed producer. The validity period is nine months from the date of test at the time of initial certification. The validity period can be further extended for six months provided on retesting the seed conforms to the prescribed standards. Every certified seed lot has a white tag for foundation seed and blue tag for certified seed. The colour of the tag is golden yellow for breeder seed and opel green for truthfully labelled seed.
Seed being a living entity is highly sensitive to ambient weather conditions viz. high relative humidity and temperature, which deteriorates its viability and vigour. Improper handling of seeds also causes mechanical injury and lower down its germination and storability. Therefore, during post-harvest processing and storage seed must be handled properly and protected from high relative humidity and temperature, insect pests and rodents.
Seed may be stored in sacks or bags for short periods. Bags may need to be

disinfected, dried and cleaned before use and should be labelled properly and stacked on wooden pallets. Storage facilities should be dry, cool, and clean disinfected, and fumigated if necessary.

**Grow-out Test:**

Grow-out test is conducted to determine genetic purity of a seed lot wherever it is a pre-requisite for grant of certificate and also on seed lots where a doubt has arisen about the genetic purity. It is compulsory to subject the hybrid seed produced through the application of CHAs' to grow-out test as a pre-requisite for grant of certificate (e.g. Hybrid wheat). Grow-out test is also done for foundation seed of other crops in the off-season by some State Seed Certification Agencies.

**Frequently Asked Questions (FAQs) and their Replies**

**Q. 1. What are breeder, foundation and certified seeds**

**Breeder seed:** Breeder seed is seed or vegetative propagating material directly controlled by the originating or sponsoring plant breeder of the breeding programme or institution and/ or seed whose production is personally supervised by a qualified plant breeder and which provides the source for the initial and recurring increase of foundation seed. Breeder seed shall be genetically so pure as to guarantee that in the subsequent generation i.e. certified foundation seed class shall confirm to the prescribed standards of genetic purity. The other quality factors of breeder seed such as physical purity, inert matter, germination etc. shall be indicated on the label on actual basis.

**Foundation seed:** Foundation seed shall be the progeny of Breeder seed or be produced from foundation seed, which can be clearly traced to Breeder seed. Thus foundation seed can even be produced from foundation seed during the production of Foundation Seed the minimum seed certification standard shall be the same for both foundation seed stage-I and II. The certification tag shall be white colour for both foundation seed stage-I and II. The production of foundation seed stage I and II shall be supervised and approved by the certification agency and be so handled as to maintain specific genetic identity and genetic purity and shall be required to confirm to certification standards specified for the crop / variety being certified.

**Certified Seed:** Certified seed shall be the progeny of foundation seed and its production shall be so handled as to maintain specific genetic identity and purity according to the standards prescribed for the crop being certified. Certified seed may be the progeny of certified seed provided this reproduction does not exceed three generations beyond foundation seed stage-I.

**Q. 2 What is Labelled Seed?**

The seed notified under Section 5 of the Seeds Act, 1966, such seed sold in the market has to be labelled as prescribed under Section 6(a) and (b) of the Seeds

Act Such seed is called Labelled Seed.

**Q. 3 What is Seed Replacement Rate?**

Seed Replacement Rate is the percentage of area sown out of total area of crop planted in the season by using certified/quality seeds other than the farm saved seed.

**Q. 4 What are the present Seed Replacement Rate for different crops?**

For the year 2003-04, Seed Replacement Rate for Wheat: 13%, Paddy: 19.16%, Maize: 24.41%, Jowar: 26.71%, Bajra: 51.02%, Gram: 7.09%, Urd: 20.48%, Moong: 19.48%, Arhar: 13.60%, Groundnut: 5.5%, Rapeseed and mustard: 66.96%, Soybean: 15.58%, Sunflower: 19.61%, Cotton: 37.25%, Jute: 68.49% for the country.

**Q. 5 What are the major schemes of Government of India in the Seed Sector?**

a) Transport subsidy on movement of seeds to Northeastern states.
b) Establishment and maintenance of Seed Banks.
c) Implementation of Plant Variety Protection Legislation.
d) Quality control arrangement on Seed and Establishment on Nationa Seed Research & Training Centre.

**Q. 6 What are Genetically Modified, transgenic crop/ seed?**

Genetically Modified seed is developed by application of biotechnology wherein a specific gene from other genus is inserted by genetic manipulation to make it resistant against certain characteristics like insect pest resistance, for example in the Bt. cotton, Cry1 AC gene has been incorporated in the cotton seed from a soil bacteria i.e. Bacillus thirugenesis which make it resistant against the attack of boll worm.

**Q. 7 . What are the penalty provisions for sale of spurious seed?**

If any person contravenes any provisions of the Seeds Act/Rules, on conviction be punishable:

a) for the first offence with fine which may extend to five hundred rupees, and

b) in the event of such person having been previously convicted of an offence under this section, with imprisonment for a term which may extend to six months, or with fine which may extend to one thousand rupees, or with both.

**Q, 8. What are the rules for export and import of seeds?**

Export/import are governed by EXIM Policy of 2002-07 issued by Ministry of Commerce. Under EXIM Policy, provision is made to import which governed by the New Policy on Seed Development, 1988 read with Plant Quarantine Order, 2003 and amendments made thereon. For restricted items the EXIM committee of DAC is empowered to take decision of import/export.

**Q. 9. What is NSC?**

The National Seeds Corporation Ltd.(NSC), a Public Sector Undertaking under the administrative control of the Department of Agriculture and Cooperation, was established in the year 1963 under the Companies Act, 1956 with the

objective of producing and distributing Seeds of high quality to the farmers. The Corporation undertakes the production of seeds through Contract Growers. NSC is dealing with about 560 varieties in 79 crops.

**Q. 10. To whom the farmers have to approach when the seed fail to perform?**

Director of Agriculture/Joint Director of Agriculture/Seed Inspector of the areas concerned.

**Q. 11. Is there any facility for the farmer to get the seed tested before sowing?**

Seed users and seed producers could get the seed sample tested in the State Seed Testing Laboratories with the minimum fee prescribed to obtain the result to be used as information for seeding, selling or labeling purposes.

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# PRINCIPLES AND PRACTICES OF SEED PRODUCTION IN CROSS-POLLINATED CROPS

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A high quality seed of improved variety is the base of modern agriculture. Production of genetically pure and good quality seed of varieties and hybrids of field, vegetable, flower and other plant species is a task requiring high technical skills and financial investment. During seed production strict attention must be given to the maintenance of genetic purity and other qualities of seeds to realize the superiority of new and improved varieties.

## Genetic Principles

Genetically pure seed can yield higher production compared to the existing varieties being cultivated by the farmers. Any impurity in the variety at genetic level leads to low yield and inferiority of other traits of importance. Impurity of a variety could arise in its genetic features. The reasons of variety deterioration are important to take up measures for quality seed production. These are listed by Kadam (1942). These are as follows:-

1. Developmental variation
2. Mechanical mixture
3. Mutation
4. Natural Crossing
5. Minor Genetic Variation
6. Selective Influence of Diseases
7. Variety development method

Among these the mechanical mixtures, natural crossing and selective influence of diseases are the most important reasons for genetic deterioration of varieties during seed production, followed by raising the seed crop in areas outside their adaptation which may cause developmental variation and genetic shifts in varieties.

### *1. Developmental variations*

A variety is usually developed in a normal growing condition and its adaptation. Seed crops are also usually taken up in such similar conditions. However, when seed crops are grown in a different environmental, soil and fertility conditions for several consecutive generations the developmental variation may arise. This changes the varietal make-up to a certain extent that genetic impurity arises over a period of time. To minimize such variations to occur in the varieties it is advisable to grow them in their areas of adaptation and growing season.

### *2. Mechanical mixtures*

This is the most important source of variety deterioration during seed production. Mechanical mixtures may often take place at time of sowing, if

more than one variety is sown with same seed drill machine. The volunteer plants of the same crop in the same field or different varieties grown in adjacent fields are potential sources of creating admixture in the resulting seed lots. Chances of varietal admixture are also possible during harvesting and threshing operations. It is more so in case of hybrid seed production. Often the seed produced of all the varieties are kept on same threshing floor, resulting in considerable varietal mixtures. Combine or threshing equipment is often contaminated with seeds of other varieties in augers, elevators etc. Further gunny bags, seed bins, elevators etc. are also quite often contaminated with seeds of other varieties. To avoid this sort of mechanical mixture contamination it would be necessary to rogue the seed fields and practice the utmost care during seed production, harvesting, threshing and further handling.

***3. Mutations***

This is not a serious factor of varietal deterioration. In majority of cases it is difficult to identify or detect minor mutations. The mutants being very few in number can be removed from seed plots during routine inspection of seed crops at various stages of its growth, flowering and seed set.

***4. Natural out-crossing***

In sexually propagated crops natural crossing is another most important source of varietal deterioration due to introgression of genes from unrelated stocks which can be only solved by prevention. The extent of varietal contamination depends upon the amount of natural cross-fertilization. The deterioration in a variety due to natural crossing occurs due to the following reasons:-

i) Natural crossing with undesirable type
ii) Natural crossing with diseased plant
iii) Natural crossing with off-type plants

In cross fertilized crops, natural crossing is the major source of genetic contamination. According to Bateman (1947) the extent of genetic contamination in seed fields due to natural crossing depends upon the following factors:

i) The breeding system of species
ii) Isolation distance
iii) Varietal mass
iv) Pollinating agent

With increased isolation distance between two varieties being grown in the same season and location the contamination generally deceases, although there may be small amounts of contamination even over wide distances. Isolation of seed crops, therefore, is a primary factor in seed production of crop plants that are cross pollinated by wind or insects. The extent of contamination depends upon the direction of prevailing winds, number of insects present and their foraging activity, humidity and temperature at the time of anthesis etc. In addition to these the mass of varieties grown within the isolation is also an important factor in the amount of influencing the degree of contamination.

***5. Minor genetic variation***

Minor genetic variation may still exist even in the varieties appearing phenotypically uniform and homogeneous at the time of their release. Selective elimination by environment may lead to loss of some of these variations during later cycle of production. To overcome these De Hann (1953) has suggested yield trials of lines propagated from plants of breeder's seed in the maintenance of self fertilized crop varieties. Minor genetic variability is more prevalent features in cross pollinated and often cross pollinated crop species. Care during maintenance of nucleus and breeder seed is necessary in such cases.

***6. Selective influence of disease***

The selective influence of diseases in varietal deterioration is also of considerable importance. New crop varieties, though are resistant to major diseases, often become susceptible to new races of pathogens and are out of seed programmes. Similarly vegetatively propagated stocks deteriorate fast if infested by viral, fungal and bacterial diseases. During seed production it is very necessary to produce disease free seeds/stocks.

***7. Variety development method***

In certain instances serious instabilities may occur in varieties due to incomplete genetic constitution/cytogenetical irregularities not properly assessed in the new varieties prior to their release. Premature release of variety still segregating for resistance and susceptibility to disease or other factors may also be important in the deterioration of varieties. This could be looked upon as the factor of the variety testing programme. In addition to the factors discussed above, other factors such as break down in male sterility, certain environmental conditions and other heritable variation may considerably lower the genetic purity.

**Maintenance of genetic purity during seed production**

The methods suggested by Horne (1953) and Hartmann and Kester (1968) may be used wholly or partly to maintain high levels of genetic purity during seed production.

The various steps suggested by Horne (1953) to maintain varietal purity, synonymous to genetic purity of a variety, are as follows:

a) Use of approved seed only in seed multiplication
b) Inspection and approval of fields prior to planting
c) Field inspection and approval of growing crops at critical stages for verification of genetic purity, detection of mixtures, weeds and for freedom from noxious weeds and seed borne diseases etc.
d) Sampling and sealing of cleaned lots and
e) Growing of samples of potentially approved stocks for comparison with authentic stocks (grow out test).

The various steps suggested by Hartmann and Kester (1968) to maintain varietal purity are as follows:

a) Providing adequate isolation to prevent contamination by natural crossing or mechanical mixtures
b) Roguing of seed fields prior to the stage at which they could contaminate the seed crop
c) Periodic testing of varieties for genetic purity
d) Avoiding genetic shifts by growing crops in areas of their adaptation only
e) Certification of seed crops to maintain genetic purity and quality of seed
f) Adopting the generation system and
g) Grow out test

The mechanism of maintaining genetic purity is further strengthened by the generation system of seed multiplication. In this system, the seed production is restricted to four generations only including nucleus seed. Starting from breeder seed, the seed can only be multiplied upto two more generations, i.e., foundation and certified. The important points for maintaining genetic purity during seed production are:-

***a) Control of seed source*:** The use of seed of an appropriate class and from an approved source is necessary for raising a seed crop. Three classes of seeds, namely breeder, foundation and certified are generally recognized in seed certification in India.

I. Breeder seed: It is seed or vegetative propagating material which is directly controlled by the originating or in certain cases, the sponsoring breeder or institution and which provides for the initial and recurring increase of foundation seed.
II. Foundation seed: Foundation seed include seed stock so handled as to most nearly maintain specific genetic identity and purity and that may be designated or distributed by an agricultural experiment station/seed producing organisation. Production of such seeds must be carefully supervised or approved by representatives of the station. Foundation seed is the source of all other certified seed classes.
III. Certified seed: It is progeny of foundation or certified seed that is so grown and handled to maintain satisfactory and desirable level of genetic identity and purity and that has been approved and certified by certifying agency.

***b) Preceding crop requirements*:** This has been fixed to avoid contamination through volunteer plants and also the soil borne diseases.

**c) *Isolation*:** The isolation of seed crops from various sources of possible contaminants is the most necessary requirement for raising seed crops. Contamination may be due to natural crossing with other varieties grown alongside and off types present in the seed fields; contamination due to

mechanical mixtures at time of sowing, harvesting, threshing, processing and handling of seeds and contamination due to seed-borne diseases from nearby fields. Protection from these sources of contamination is necessary for maintaining genetic purity and good quality seed.

**d) *Roguing of seed fields*:** The occurrence/presence of off- type plants, i.e. plants differing in their characteristics from those of the seed variety is another potent source of genetic contamination. The removal of such plants from the seed plot is referred to as roguing. There are three main sources of off - type plants. The off-type plants may arise due to presence of some recessive genes in homozygous conditions at time of development and release of varieties. The recessive genes may also arise by mutation. The heterozygous plants segregate for the characteristics governed by the particular gene in later stages of multiplication cylces and give rise to off-types. Another source of off-type plants is the volunteer plants arising from accidentally planted seeds or from seeds produced by previous varieties grown in the same plot. For this reason the fields for producing seed of a particular variety should not have been grown with a potentially contaminating variety for a specified number of preceding years. Off-type individual plants should be rouged out of seed production fields before pollination occurs. Regular supervision by trained personnel is imperative. Also it may be necessary to control certain seed- borne diseases.

**e) *Seed certification*:** The genetic purity in commercial seed production is often maintained through a system of seed certification. To accomplish this, qualified and well experienced personnel of seed certification agency carry out field inspections at appropriate stages of crop growth. They also make inspections to verify that the seed crop/seed lot is of the requisite genetic purity and quality, after harvesting to verify quality and at the processing plants draw samples for seed testing and sometimes for grow out test also. The field standards include land requirement, isolation requirements maximum permissible off-type plants etc.

**f) *Grow-out test*:** Varieties being grown for seed production should periodically be tested for genetic purity by grow-out tests, to make sure that they are being maintained in their true form.

**Variety Maintenance and Nucleus Seed Production of Open Pollinated Varieties**

In case of cross pollinated or often cross pollinated crops, the following scheme is followed for variety maintenance:

1) Single plants typical of the variety are selected from a seed crop plot.

2) These selected single plants are harvested and threshed individually. The threshed seed is examined critically for colour, shape and size etc. The seed packets that are not true representative of the variety are rejected. The selected individual plant seed is divided into two parts.

3) In the next season, one part of the seed from selected plants is sown in plant rows. The remaining seed (other part) of each plant is stored. It is very important to maintain identity of the plant row and the reserve seed.
4) Plant progeny rows are examined critically throughout the growing season. Any row with one or more off type plant is rejected as when detected.
5) At the time of flowering the plants should be examined daily or at alternate days to minimise contamination from off type plants. All the rejected rows should be removed at the earliest after detection.
6) Plant rows true to the variety for morphological characters are retained. These rows are harvested separately.
7) Seeds from individual plant progeny rows is again examined for colour, shape, and size etc. Seeds of the plant rows not conforming to the variety are rejected.
8) Reserved seeds of single plants which produced true to type progenies are bulked to constitute the nucleus seed.
This bulked seed is planted in isolation for further multiplication or breeder seed production.
Since this procedure is laborious and difficult to practice, it is followed mainly for purification of varieties. For annual maintenance, breeders select a large number of true to type plants from central part of the breeder seed production plot and bulk seed of these selected plants to constitute nucleus seed. This method is also known as mass selection. This may be practised once the variety has been purified.

**Maintenance of Inbred Lines**

Inbred lines are maintained by selfing of representative plants or by sib mating between plants grown in a plant to progeny row after thorough rogueing for plant type and other morphological characters. Continuous selfing to maintain the inbred line causes inbreeding depression in the characters. On one side selfing helps in maintaining the inbred line and on the other side sib mating tends to prevent excessive loss of vigour. Therefore, selfing and sib mating in alternate generations are suggested. In case of male sterile line (A Line) ,it is maintained by crossing with male fertile counterpart (B Line). The representative plants of A line are hand pollinated with bulk pollen collected from B line. Occasionally paired crosses between selected plants of A and B line are made. Their evaluation is done in the next season and true plants are used for bulk seed production of A line. Also A and R line are evaluated to ensure near 100 % male sterility in A line and near 100% fertility restoration in resulting hybrid.

## AGRONOMIC PRINCIPLES AND PRACTICES

Apart from following the genetic principles the application of the agronomic principles are equally important for quality seed production. Seed production differs from commercial crop production in several aspects. Special methods

and precautions are needed to produce seed of desired quality. These are as follows:

**a) Selection of a suitable agro-climatic region:**

A crop variety to be grown for seed production in an area must be adapted to the photoperiod and temperature conditions prevailing in that area. The crop varieties sensitive to photoperiodism and temperature should be grown in selected regions where these could be economically produced. Regions of moderate rainfall and humidity are much more suited to seed production than regions of high rainfall and humidity. Most crops require a dry sunny period and moderate temperatures for flowering and pollination. Excessive dew and rain cause hindrance in normal pollination resulting in poor seed set. Similarly very high temperature causes desiccation of pollen resulting in poor seed set. If hot dry weather conditions prevail particularly during flowering period, many crops such as vegetables, legumes and fruit trees fail to set seed effectively and produce many seedless fruits. The crops invariably require cool conditions with low atmospheric humidity to flower and pollinate normally. In wind pollinated crops bright sunny weather with gentle winds which cause an even flow of pollen during flowering is conductive to effective pollination and good seed set. Similarly very cold temperatures may also damage seed quality especially in the early phases of seed maturation. In general, regions with extreme summer heat and very cold winters should be avoided for seed production unless particular crops are especially adapted to grow and produce seed under these conditions. Apart from affecting pollination, excessive rainfall leads to a higher incidence of diseases and makes seed harvesting extremely difficult. It may also result in delayed maturity and pre- germination of seed in many standing crops. As the seed crop approaches maturity it becomes increasingly susceptible to shattering. Strong winds and heavy rainfall at or near harvest time may cause heavy seed losses particularly in crops which have a tendency to shatter their seed readily. Such conditions may also complicate the harvesting and post-harvesting operations. It is, therefore, necessary to ensure ample sunshine, relatively moderate rainfall and the absence of strong winds for productive and high quality seed production and these must be kept in view in the selection of areas for seed production.

**b) Selection of seed plot:**

The plot selected for seed crop must have following characteristics:-

- Soil texture and fertility of plot should be according to the requirement of the seed crops.
- The seed plot should be free from volunteer plants, weed plants and other crop plants.

- The soil of the seed plot should be comparatively free from soil-borne diseases and insect pests.
- In the preceding season the same crop should have not been grown on this plot,
- It should be feasible to isolate the plot as per requirements of certification standards.

**c) Isolation Requirement:**

The seed production plot should be isolated from various sources of contamination by a certain minimum distance, known as isolation distance. Isolation of seed plots from contamination sources is more important for cross pollinated crops to prevent pollen- mediated genetic contamination. The contamination source may be volunteer plants in the seed field, other variety in nearby field or same variety not conforming to genetic purity and other cross compatible crop/weed species. Isolation distance depends upon following factors:

***i) Mode of reproduction of seed crop***: Cross pollinated and often cross pollinated crop varieties require more isolation (up to 1000m) as compared to self pollinated crops (3-10m).

***ii) Pollinating Agents****:* In cross pollination, pollen has to be transferred from one plant to other plant through some agents which may be wind, insect, gravity etc. In case of anemophilous crops, direction and velocity of wind is very important e.g. Sorghum, maize, pearl millet. The size of the insect population and its foraging behaviour are the major factors in entomophilous crops. The pollinating agents are useful in properly isolated plots for hybrid seed production but become a nuisance in maintaining genetic purity if isolation distance from contaminant source is not adequate.

***iii) Mass of pollen:*** It is also a very important factor by itself and associated with isolation requirement. If area of the seed crop is small, even a little cross contamination may render the seed lot unfit as it may not meet the minimum standards of purity. In large seed production plots, even if some cross pollination from undesirable type takes place, it gets diluted in the large seed lot and the purity status of the lot may remain higher than the minimum standard. It is always desirable to take up seed crop of OPVs and synthetics and composite varieties of cross pollinated nature in a considerably larger size so that there is sufficient random mating among the plants in the field. This factor may very well be taken care of in compact area approach of seed production.

***iv) Differential Blooming Dates*:** When the seed crop and contaminant plants flower at the same time, proper space isolation is must. But when they flower at different times, there is no need of space isolation. This isolation is known as time isolation. It is permitted in only non-tillering crops like maize provided that

5% or more of the plants in seed parent plot do not have receptive silks when more than 0.2% of plants in adjacent field are shedding pollen.

***v) Presence of Border Rows:*** In hybrid seed production of maize, isolation requirement is 200m from any maize with same kernel colour and texture. This can be reduced by planting border rows of male parent all around the seed parent. The number of border rows to be planted is determined by size of seed field and its distance from the contaminant source. An interval of 25-30 days between blooming of two varieties is adequate for time isolation.

**Isolation requirements for different seed crops**

| **Crop** | **Isolation distance (m) for** | |
|---|---|---|
| | **Foundation seed** | **Certified seed** |
| Maize (inbreds, foundation & single cross) | 400 (600) | --- |
| Maize (composites, synthetics, OP ) | 400 | 200 |
| Maize (Hybrids) | --- | 200 (300) |
| Sorghum (OP variety) | 200 (400) | 100 (400) |
| Sorghum (Hybrids) | 300 (400) | 200 (400) |
| Pear millet (Comp, Syn & OP) | 400 | 200 |
| Pear millet (Hybrids) | 1000 | 200 |
| Pigeon pea | 200 | 100 |
| Sunflower | 400 | 200 |
| Castor | 300 | 150 |

*The figures in the parenthesis are isolation distance from different kernel coloured plants and teosinte in case of maize; and from Johnson grass and high tillering forage sorghum with grassy panicle in case of sorghum.*

Keeping all the above factors in mind, if the seed crop is sufficiently isolated from source of contamination, the possibility of deterioration of a variety through natural out-crossing can be almost eliminated.

**d) Land preparation:**

The land for seed crop must be prepared well. Good land preparation helps improved germination, good stand establishment and destruction of potential weeds. It also aids in water management and good uniform irrigation.

**e) Selection of variety:**

The variety for seed production must be carefully selected. Except in exceptional cases it should satisfy the following criteria:

- The variety should be adapted and recommended to the agro-climatic conditions.
- The variety should be high yielder in that particular growing condition.
- The variety should posses other desirable attributes, namely disease resistance, grain quality etc.

**f) Seed source:**

The seed used for raising a seed crop should be of known purity, appropriate class and obtained from an authorized source. While buying the seed the following factors should be carefully examined:

- The seed of the appropriate seed class is bought for raising a foundation seed crop, seed of the breeder seed class is required and for raising a certified seed crop the seed of the foundation seed class is required for sowing.
- The tag and seals of the breeders/foundation seed bags purchased are intact.
- The validity period has not expired.
- All the bags are of the same variety.

**g) Seed treatment:**

The seed may require seed treatment before planting, if they are not already appropriately treated. Depending upon the requirement one or more of the following seed treatments may be given:

- Chemical seed treatment.
- Bacterial inoculation for legumes.
- Seed treatment for breaking dormancy/ hardseededness.

**h) Time of planting:**

The seed crops should invariably be sown at their normal planting time. Depending upon incidences of disease and pests, some adjustments could be made, if necessary. At time of planting there should be sufficient soil moisture for germination to take place.

**i) Seed rate:**

Lower seed rates than those for raising commercial crop are desirable because they facilitate roguing operations and inspection of seed crop.

**j) Method of sowing:**

The seed crops should be sown in rows with the exception of thickly sown crops where the sowing could be done by broadcasting. The most efficient and ideal method of sowing is by mechanical drilling, as it allows the deposit of the seeds in desired amount at uniform depth. It is of utmost importance to ensure that the seed drills/planters are absolutely clean, i.e. free from leftover seeds of other crops. The sowing of seed crops in rows helps in conducting effective plant protection measures, roguing operations and field inspections. For many crops spacing within row is often more important than distance between rows, close spacing in row usually stimulate fibre and oil crops to branch more profusely at top and produce more seeds. For hybrid seed production

planting of two parents namely, female and male parent has to be taken up in a definite proportion. It is imperative to ensure that the seeds of male and female parent line do not get mixed while planting. After planting of the male and female lines six to eight border rows of the male parent may also be sown, if required. After sowing male rows should be marked appropriately to facilitate further operations. The synchronization of flowering should also be ensured (through staggered sowing or other means) to increase availability of desired pollen load when stigma is receptive. Direction of rows is also important for hybrid/female line seed production in case of wind pollinated crops and it should be kept across (perpendicular to) the wind direction prevailing at the time of flowering.

**k) Depth of sowing:**

Depth of sowing is very important in ensuring a good plant stands. Small seed should be usually planted shallow, but larger seeds could be planted a little deeper. Seeds would emerge from greater depths in sandy soils than in clay soils and also in warm soil as compared to cold. In dry soils seeds should be planted slightly deeper as so that they come in contact.

**l) Supplementary pollination:**

Provision of honey bees in hives in close proximity to the seed fields of crops mainly for cross-pollination ensure good seed set and thereby greatly increase seed yields.

**m) Weed control:**

Good weed control is a basic requirement in producing good quality seed. Weeds may cause contamination of the seed crop in the following ways in addition to reduction in yield:

- The presence of weed seeds at time of crop harvest leads to mixing of weed seeds with crop seeds. In many instances it is difficult to remove them during the processing of seeds.
- The presence of weeds in the seed field or nearby areas may serve as host to no. of diseases. Good and effective weed control, therefore, would be necessary to obtain good seed yields and to avoid contamination.

**n) Disease and insect control:**

Successful disease and insect control is another important factor in raising healthy seed crops. Apart from reduction in yield, the quality of seeds from disease and insect damaged plants is invariably poor. Poor disease and insect control affects seed quality in the following three ways:-

- There are a number of diseases which are systemic. If these are not checked the seed produced will get infected with spores of such diseases and produce diseased plant in next season.
- There are certain diseases which are not systemic, leave their spores on seed coats. If not checked this results in a greater susceptibility to various seedling diseases which can affect the crop.
- Seed yield and quality are reduced.

Following principles may be adhered for an effective management of diseases and pests in seed crops:-

- Sow only treated seed.
- Prepare and adopt appropriate schedule of spraying for effective disease and insect control.
- Roguing of diseased plants and ear heads from time to time also helps check the further spread of diseases.

**o) Nutrition:**

Nitrogen, phosphorous potassium and several other elements play an important role for proper development of plants and seeds. It is therefore advisable to know and identify the nutritional requirements of seed crops and apply in adequate quantity. Adequate fertilization results in maximum yields, good seed quality and better expression of plant type which facilitate roguing and thereby helps maintain higher genetic purity as well.

**p) Irrigation:**

A relatively dry regions are more suitable for quality and disease free seed production. In such regions irrigation is essential to grow a good crop to obtain good seed yields. The irrigations may be required before planting and at intervals upto flowering. One or two irrigations beyond flowering are desirable for seed crop.

**p) Roguing:**

Roguing in most of the field crops may be done at the following stages as per needs of the seed crop.

- Vegetative /pre-flowering stage
- Flowering stage
- Maturity stage

Any plant not conforming to the typical characteristics of the variety is an off-type and removal of such plants is called rogueing. It is one of the most important operations in seed production to maintain genetic purity. The timing of rogueing is another important aspect particularly in cross pollinated crops. The off-type plants may arise because of admixture, segregation, natural out-crossing, volunteer plants and mutations. They may be with respect to any

morphological character such as height, growth habit, flowering time, leaf colour, shape, size and colour of flower, pod/fruit colour etc.

- In case of cross pollinated crops the off-types must be removed before flowering. Off-types detected during flowering should be removed immediately and for that rogueing should be carried out very frequently to minimise out-crossing. Rogueing by a particular individual(s) should be restricted to only one variety in a day to avoid cross contamination with pollen sticking to clothes and body parts of the personnel doing rogueing. Rogueing should not be very strict in case of composites, synthetics and open pollinated varieties to maintain their broad genetic base.
- In case of hybrid/male sterile line seed production, pollen shedders should be removed daily in the morning before they shed pollen. It will reduce frequency of pollen shedders in A (female) line seed in the next year and A line plants in the resulting hybrid. All other plants not conforming to the variety, other crop plants, objectionable weed plants as well as disease infected plants should also be removed. All the plants appearing outside the rows/lines may also be removed as they are volunteer plants. In general, as a rule, all the rogued plants should be uprooted and taken away from the seed plot and be destroyed.

**q) Field Inspection:**

Foundation and certified seed are produced under supervision of State Seed Certification Agency. The seed plots are inspected by trained personal authorised by the agency. Number of field inspections may vary from two to four. Field inspection meant to verify those factors which can cause irreversible damage to the genetic purity or seed health shall be conducted without prior notice to the seed producer. Though breeder seed has been kept out of purview of certification, officials of certification agency and National Seeds Corporation are associated with breeder seed production. The breeder seed crop is monitored by a joint monitoring team consisting of breeder of the variety, producing breeder; and representatives of SSCA and NSC. In case of maize, ears are inspected after maturity/harvest and should not have in excess of 0.20%, 0.50% and 1.00% off type ears including ears with off type kernel for inbreds/foundation single cross, hybrids and open pollinated varieties, respectively.

**r) Harvesting of seed crops:**

***Time of harvest***: The optimum time of harvest is when the seed is fully mature with optimum moisture content, when weather damage has just begun and the seed is easily harvested and cleaned resulting in minimum harvest losses. Harvesting at earlier stages makes combining difficult and relative losses due to

threshing and cleaning are greater. Similarly harvesting at a late stage may result in increased weather damage to seeds and losses due to shattering seeds and lodging of plants in the field.

***Method of harvesting***: In India harvesting of crop is done by hands. This if done at appropriate time gives good quality of seed. However, the subsequent quality of seed is dependent upon the handling of harvested crop and the care taken during threshing. Every effort should be made to avoid chance mechanical mixing, mechanical injury to seed during threshing. Lot identity should be maintained. If harvesting is done with combine and the seed crops are directly combined in the fields, threshing is not required. Precautions must be taken to adjust the combines properly so as to keep various losses and mechanical injury to seed during combining.

In hybrid/MS line seed production, where two parents are used, harvesting requires special attention. All the male parent rows should be harvested first and moved away from the field. The plot is then inspected for presence of any male parent plants. The female parent rows are then harvested as hybrid seed.

**s) Drying of seeds:**

The seed lots are usually are at high moisture content at the time of harvesting and threshing. In order to preserve seed viability and vigour it is necessary to dry seeds to safe moisture content levels. Drying of seeds to safe moisture limits should be done quickly. If the seeds are to be artificially dried they should be supplied to processing plants soon after harvesting.

**t) Seed Testing and Labeling:**

A representative sample of the processed seed lot is sent to a notified Seed Testing Laboratory for analysis of its quality. The seed lot is approved by the certification agency and tags are issued with a satisfactory test report. The seed is then packed in bags of desired quality and size. The packed seeds need attention during storage periodically.

**Seed Standards for Genetic Purity:**

All the certified seed lots should conform to the following minimum standards for genetic purity unless and otherwise prescribed.

| ***Class*** | ***Minimum genetic purity (%)*** |
|---|---|
| a) Foundation | 99.00 |
| b) Certified | |
| i) Open pollinated varieties, composites, synthetics | 98.00 |
| ii) Hybrids | 95.00 |

***a)*** **Grow-out test:** Grow-out test is conducted to determine genetic purity of a seed lot. Hybrid seed of cotton and castor is subjected to GOT before it is finally approved as certified seed. Hybrid seed produced through application

of CHA to the female parental line is also compulsorily subjected to GOT. The certification agency may also conduct GOT where a doubt has arisen about the genetic purity.

***b)*** **Seed storage:** The best method of storing seed for short period is in stacks or bags in ordinary building or godowns. After sun drying the seed should be filled in neat and clean bags. If old bags are to be used they should be properly cleaned and treated before use. Each and every bag should be marked appropriately. The stacks of bags should be made on wooden pallets. The godowns to be used for storage should be dry, cool, clean and sprayed with malathion and later fumigated as and when necessary.

# SEED PRODUCTION IN WHEAT AND BARLEY

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Wheat is the second most important crop after rice in India and occupies approximately 29 million ha area. Bread wheat (*Triticum aestivum*) is the main species; occupying about 90-95 percent of total area. It is grown mainly in the northern and central part of the country. Variety development program is very strong in wheat and new varieties are developed every year. Quality seed is very important along with agronomic practices to realize worth of an improved variety. Availability of quality seed in sufficient quantity year after year is a must for enhancing agricultural production. Quality seed may be defined as seed of an improved variety which is high in genetic and physical purity, has high germination and vigour; is free from weed seeds and seed borne diseases, and has low moisture content. There are three classes of seed in India, namely breeder seed, foundation seed and certified seed. Breeder seed is produced by the breeder or the breeding Institute; and is used to raise foundation seed crop. Certified seed is produced mainly from foundation seed and is grown for commercial cultivation.

Seed production differs from crop production in several aspects. Both wheat and barley have similar pollination behavior as well as growth habit. So their seed production techniques are also similar. In seed production of wheat and barley the following points should be taken care of.

**Land requirement:** The plot selected for seed production should be fertile, well drained and free from volunteer (self sown) plants.

**Isolation requirement:** Wheat and barley are self pollinated crops. So isolation requirement is merely 3 meters for foundation as well as certified seed. The isolation is mainly to avoid admixture from adjoining plots. The isolation requirement is 150 meters from loose smut infected plots of wheat, triticale and rye (Table-1).

**Table-1:** Field Standards: Isolation requirement.

| **Contaminants** | **Minimum distance (m)** | |
|---|---|---|
| | **Foundation** | **Certified** |
| Field of other variety/same variety not conforming to varietal purity requirements for certification | 3 | 3 |
| Fields of wheat, triticale and rye with infection of loose smut in excess of 0.10% and 0.50% for foundation and certified seed respectively. | 150 | 150 |

| | | |
|---|---|---|
| Fields of barley with infection of loose smut in excess of 0.10% and 0.50% for foundation and certified seed respectively. | 150 | 150 |

**Seed source and method of sowing:** Seed used for sowing of seed crop, should be of appropriate class and be procured from a reliable source. The seed crop should always be planted in lines with the help of a seed drill/planting machine. Roguing lanes (empty rows at intervals) should be left to facilitate rouging, cultural operations and field inspection. The line sowing of the crop also helps in detection of volunteer plants in the space between two lines. The seed drill must be thoroughly cleaned before and after sowing of one variety to avoid mechanical mixing. If possible, other crop should be sown in between two varieties

**Agronomic practices:** Cultural practices for the seed production are usually the same as recommended for crop production. All recommended agronomic practices should be followed for optimum growth and proper expression of plant type. Presence of *Phalaris* plants in the wheat and barley seed crop becomes hindrance in rogueing. Recommended doses of Phosphorus and Potassium should be applied; however, nitrogen may be slightly reduced to avoid lodging. Rogueing and inspection are very difficult in a severely lodged crop and hence the seed crop may not be approved.

**Roguing:** Any plant not conforming to the typical characteristics of the variety is an off type and removal of such plants is called 89ouging. It is one of the most important operations in seed production to maintain genetic purity. The off type plants may arise because of admixture, segregation, natural out-crossing, volunteer plants and mutations. The variants may be with respect to growth habit, height, leaf, ear, awn characters etc. All off-types, other crop plants, objectionable weed plants as well as disease infected plants should be removed time to time. Entire plant (all tillers) should be uprooted during rouging and rogued plants should not be left in the seed crop, rather they should be thrown away.

**Field inspection:** In India seed standards have been prescribed for foundation and certified seed only. There are two type of standards i.e. field standards and seed standards. The field standards apply to the seed plots and standing crops while seed standards are applicable at seed level (seed lots). Field standards include land requirement, isolation requirement, maximum permissible level of off type, inseparable other crop plants, plants infected by seed borne diseases etc. Foundation and certified seed are produced under supervision of State Seed Certification Agency. Field inspection is done by SCA to ensure that the

seed crop conforms to the minimum field standards. However, breeder seed has been kept out of purview of certification. The breeder seed crop is monitored by a joint inspection team consisting of breeder of the variety, producing breeder; and representatives of SSCA and NSC.

**Table-2:** Field Standards: Specific requirements (varieties)

| **Factor** | **Maximum permitted (%)*** | |
|---|---|---|
| | **Foundation** | **Certified** |
| Off-types | 0.050 | 0.20 |
| Inseparable other crop plants | 0.010 | 0.050 |
| Plants affected by seed borne diseases | 0.10 | 0.50 |

* Standards for off-types and inseparable other crop plants shall be met at the final inspection and for loose smut be met at any inspection conducted between ear emergence and harvesting..

**N.B.** A minimum of two inspections from time the crop approaches flowering until it is ready for harvesting.

**Harvesting, threshing and processing of seed:** The crop should be harvested after maturity at a proper moisture content to minimize damage to the seed. The outer 8-10 rows should be left as grain and harvested at the end as they may have some out crossed plants. The combine harvester should be thoroughly cleaned otherwise it may become the major source of contamination and will nullify all care and efforts made in the field. Proper care should also be taken to avoid admixing during seed processing.

**Seed testing, labeling and storage:** A representative sample of the processed seed is sent to the notified seed testing laboratory for analysis. Seed testing is done as per the ISTA rules. If the seed lot meets the prescribed seed standards (Table-3), the lot is approved and labels and certificates are issued (by the SSCA) to the seed producer. In case of breeder seed, since no genetic purity standards are prescribed, it should be as pure as possible; in no case less than that of foundation seed (99.00%). The other quality factors such as physical purity, germination etc. is indicated on the label on actual basis. Label colour for breeder seed is golden yellow while for foundation and certified seeds it is white blue colored respectively.

The seed is a living entity and is sensitive to weather conditions viz. temperature and relative humidity (RH). High temperature and RH deteriorate the seed quality and shorten its life (viability and vigour). So the seed should be stored at low temperature and relative humidity conditions; and be protected from storage pests, mainly insects.

**Table-3:** Seed Standards

| **Factor** | **Standards** | |
|---|---|---|
| | **Foundation** | **Certified** |
| Pure seed (Minimum) | 98.0% | 98.0% |
| Inert matter (Maximum) | 2.0% | 2.0% |
| Other crop seeds (Maximum) | 10/kg | 20/kg |
| Total weed seeds (Maximum) | 10/kg | 20/kg |
| Other distinguishable varieties (Maximum) for barley | 10/kg | 20/kg |
| Objectionable weed seeds (Maximum)* for wheat | 2/kg | 5/kg |
| Seeds infested by Ear-cockle & Tundu (Maximum) for wheat | None | None |
| Seeds infested by karnal bunt (Maximum) for wheat | 0.050% (by number) | 0.250% (by number) |
| Germination (Minimum) | 85% | 85% |
| Moisture (Maximum) | 12.0% | 12.0% |
| Moisture for vapour proof containers (Maximum) | 8.0% | 8.0% |

* Objectionable weeds shall be: *Hirankhuri* (*Convolvulus arvensis*) and *Gulli Danda/ Kanaki* (*Phalaris minor*)

# PRINCIPLES AND PRACTICES OF SEED PRODUCTION IN MAIZE

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Maize (*Zea mays* ssp. *mays* L.) occupies an important position in the world economy, and serves as important source of food and feed. Together with rice and wheat, it provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries. In India, maize is an important cereal too, and grown on an area of 9.2 million hectare with production of 24.2 million tonnes. The demand for cereals will continue to increase as a consequence of the expanding human population. The world will have around 7.7 billion people by 2020, and it will reach up to 9.3 billion by 2050, and the demand for maize between now and 2050 will double in the developing world. By 2025, India too would require to double the production (50 million tonnes) of maize grain to meet the domestic demand. Being a cross-pollinated crop, high percentage of seed replacement is required in maize every year particularly for hybrids to meet the growing demand. Thus, maintenance of inbreds and production of good quality hybrid seeds is of paramount importance.

## Floral biology

Maize is cross pollinated crop and seed production of a hybrid involves crossing a female parent with a male parent under isolation. Being a monoecious plant, male and female inflorescence is borne on separate part of a single maize plant. The main shoot terminates into male inflorescence which is made up of a central axis and several spreading rachis and is referred to as '*tassel*'. The female inflorescence is called as '*silk*' and it develops into the ear. The ear is enclosed within several layers modified leaf sheaths called as '*husks'*. Maize is generally protandrous in nature, where the male flower matures before female flower, generally with a gap of 2-3 days. However, with induction of moisture stress the anthesis silking interval (ASI) increases, and in extreme conditions silk may not emerge at all. Monoecism and protandry lead to wind mediated cross pollination in maize

## Type of hybrids

There are number of possible type of hybrids in maize *viz.*, single cross- (A × B), three way cross- [(A × B) × C], double cross- [(A × B) × (C × D)], top cross- (A × OPV) and double top cross- [(A × B) × OPV] hybrid. These hybrids differ in the type of parents used in crossing programme, their parental composition but, in all cases, the hybrid seed sold to farmers is a cross between two or more parents used as female and male. Among all types of hybrids, single cross hybrids are generally more heterotic and uniform than other form of hybrids,

and 25-30% of the area is covered under single cross hybrid cultivation. In developed nations nearly 100% of maize area is planted with single cross hybrids.

Maize hybrids are derived from inbreds which are resulted from repeated selfing (inbreeding) of a particular maize population to generate inbreds with fixed and uniform genetic composition. Thus, all the plants of a particular inbred line are genetically identical but distinct from other inbred lines. Though maize is bisexual in nature, specific inbreds are used as male parent, while inbreds with special characteristics are used as female parents. Because of continuous selfing (inbreeding), inbred lines are usually less vigorous and low-yielding than open pollinated maize plants, but hybrids developed from two distant inbreds generally outperform the inbreds, the phenomenon is known as heterosis. Plant breeders develop and maintain the male and female parents to produce hybrid seeds every year. The desirable phenotypic features of male and female parents are as follows:

***Male/ pollen parent:*** Pollen grains of maize are light in weight and very small which is barely visible to the naked eye. Each anther contains about 2000-7500 pollen grains depending upon genotypes and approximately $14 \times 10^{-6}$ pollen grains could be produced by each tassel. The height of the male parent should ideally be taller than the female parent for easy dispersal and movement of pollen. The tassel should preferably be of lax type with long main branch with few secondary branches; and it should produce abundant pollen for a longer period. The amount of pollen produced is directly related to the cost of cultivation of hybrid seeds as lesser male row would be required to pollinate female rows.

***Female/ seed parent:*** The female parent should have good plant architecture with high yield potential. Erect and stay green leaves with productive cob bearing high number of seeds is desirable. It should have longer silk receptivity with complete exertion of cobs. The grain should be bold with attractive grain colour.

**Pollination and fertilization**

Being a protandrous crop, pollen-shedding starts 2-3 days before the emergence of silk and it continues up to 7-10 days depending on the genotypes. Pollen grains remain viable for 1-2 hours and under favourable condition of cool temperature and high humidity, it may be viable for longer period. Generally 2-3 days of ASI is most favourable for good seed set. Under stress conditions, the ASI increases leading to poor seed set hampering the seed production. During pollination, pollen grain lands on the silk, germinates and enters the embryo sac within 12-28 hours. Owing to the light weight of pollen, maize pollen is easily carried by wind aiding in easy pollen dispersal in the seed production plots.

The management of both the parents are important and requires adequate attention during seed production. The key factors that determine the successful and quality hybrid seed production are as follows:

- Purity of the female and male parent
- Ratio of female parent to male parent in the seed production field
- Removal of off-types and rogues to prevent the contamination
- Detasseling of the female parent at appropriate time
- Synchronisation of silking in female and pollen shedding in male parent
- Careful and separate harvesting of female and male parent rows to avoid mechanical seed admixture.

**Hybrid seed production**

At the time of initial release of a variety, the breeder possesses only a small quantity of nucleus seed. It has to be multiplied through few generations to produce in large quantity. Normally, genetic purity declines with succeeding crop generations. Minimum genetic purity followed in India for foundation seed is 99%, while the same for certified seed is 98% (composites) and 95% (hybrids). Hybrid seed production of single cross maize hybrid is undertaken in system of successive stages, *viz.*, breeder seed to foundation seed, and foundation to certified seed. The very first task is to multiply the nucleus seed (produced by the original breeder) to breeder seed of the inbreds, strictly under isolation to ensure genetic purity by the original/ sponsored breeder. The quality and genetic purity of breeder seed is ensured by breeder, and inspected by monitoring team at different stages of crop growth. The breeder seed is then multiplied to produce foundation seed which is further multiplied into certified seed of single cross hybrid. For production of certified seed of three way and double cross hybrid, the respective parental lines are multiplied in isolation in breeder seed stage, and single cross is produced in foundation stage followed by certified seed production of seed production hybrid.

In hybrid seed production plot, depending on the pollen dispersal capacity of pollen parent and nature of seed parent, ratio of male and female row can vary considerably. For single cross hybrid, the ratio of female rows to male rows in the field is usually 3:1, whereas it may extend up to 8:1. Tassel of the female plant is removed before initiation of pollen shedding, so that the pollen of male parent is used in the seed production plot. Removal of tassel as soon as it emerges out of flag leaf is called detasseling and is necessary to avoid selfing in female rows. Sufficient care should be taken to mark the male rows to avoid confusion during detasselling. It should be repeated daily in all weather conditions at a fixed time. Proper care should be taken during detasseling to remove the entire tassel from the female rows before anthesis of florets in the tassel. Flag leaf should not be damaged while detasseling as it may lead to reduction in yield or attack of disease. The removed tassel should be placed on

the ground, and carrying them in hand may cause contamination on receptive silks in the female rows. Use of CMS-based hybrid system is highly preferred as detasseling is not required in the female lines. Presently hybrids (used for grain purpose) do not possess CMS system; however for baby corn purpose male sterility is being used.

**Isolation distance**

Maize being a cross pollinated crop and is highly prone to contamination from foreign pollen; hence proper isolation distance from other maize field is required for production of genetically pure seed. Isolation can be taken in two ways *viz.*, spatial and temporal isolation to maintain purity during seed production. Pollen from undesirable sources can be excluded by maintaining 400m isolation distance of the seed production plot, which is the minimum seed certification standard for maize seed production. Number of isolation required for different type of hybrids can vary from 5-7 depending on the number of parents involved. On the other hand, under circumstances where spatial isolation is not possible, the same can be achieved through adjusting sowing time between two plots differing by at least 40 days difference.

**Table 1**: Number of isolations required for different types of hybrid in seed production

| S. No. | Type | Breeder Seed | Foundation Seed | Certified Seed | Total |
|---|---|---|---|---|---|
| 1. | Single cross hybrid | 2 | 2 | 1 | 5 |
| 2. | Three way cross hybrid | 3 | 2 | 1 | 6 |
| 3. | Double cross hybrid | 4 | 2 | 1 | 7 |
| 4. | Top cross hybrid | 2 | 2 | 1 | 5 |

**Cultural practices**

- The area selected for seed production should have similar climatic condition (temperature and photoperiod) as that area where the variety is intended to be recommended for cultivation. The area should have mild and dry weather with abundant sunshine. During seed production especially flowering, temperature, relative humidity, and the wind velocity should range between 25-30ºC, 60-70% and 2-4 km/hr, respectively.
- The field selected for seed production should be well drained, levelled with fertile soil preferably sandy loam to loamy soils as maize is very sensitive to water logging and drought. In addition, it should be free from pest and diseases incidence, volunteer plants, weed seeds, off types, diseased plants, soil borne pathogens. The field should not have maize in previous one season.

- The optimum time of sowing during *kharif* (rainy season) is first week of July. Heavy rainfall leads to pollen wash thereby affecting seed production. During *rabi* (winter season), sowing during first to third week of November is ideal. The flowering should coincide with mild environment, as very low temperature affects anther mortality, whereas high temperature leads to tassel blast affecting the pollen viability. Harvesting also should not coincide with rain as it affects drying, shelling and seed viability.
- The field selected for maize should have well drained and fertile soil with sandy loam to clay texture. Weed and disease and pest free soil is desirable. In order to maintain the genetic purity, the field should not have maize as previous crop. Ideally minimum of 400 m (fields of maize with same kernel colour and texture, fields of same inbred line not conforming to variety purity requirements of certification) and 600m (fields of maize with different kernel colour and texture and teosinte) distance is required to avoid any contamination by foreign pollen from the adjacent fields.
- To control seed borne pathogens, seeds should be treated with Carbendazim or Thiram at 2.5 g/kg of seed.
- The seeds may be planted on ridges or flat beds but ridges are preferable in rainy season. Depending on the plant type the row-to-row and plant-to-plant spacing can be maintained between 60 or 75 cm and 25 or 20 cm, respectively. Sowing of border rows with male parent around the field ensures production of genetically pure seeds by providing adequate pollen load and reduce contamination by foreign pollen. The seed rate depends on size and test weight of seed, plant type and male: female ratio. Generally, 15 kg/ha seed for female and 10 kg/ha seeds for male is recommended.
- The success of seed production depends on the planting of seed production potential of female parent, synchrony of male: female parent, male to female ratio, pollen shedding potential and duration of availability of pollen in male parent, and number of cobs, seeds per cob and duration of silk receptivity in female parent is vital for successful seed production. In general the male: female ratio followed is 1:2 or 1:3 or 1:4. Even ratio of 1:5 to 1:6 can be adopted if sufficient pollen load can be maintained for proper pollination and seed setting.
- Thinning must be done at 10-15 days after sowing for providing each plant space for proper growth. This also helps to realise the complete expression of the genotype. Around 12-15 days after sowing off type and extra plants should be removed. Further, dissimilar plants should be removed at knee high and flowering but before anthesis stage.

**Table 2:** Maximum limits for off types in hybrid seed production

| S. No. | Off type | Max. permitted (%) |
|---|---|---|

| | | |
|---|---|---|
| 1. | Off type plants that have shed or are shedding pollen in male parent at any one inspection during flowering when 5.0% or more of the plants in the seed field have receptive silks | 0.50 |
| 2. | 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 |
| 3. | Total of pollen shedding tassels including tassels that have shed pollens for all three inspections conducted during flowering on different dates | 2.0 |
| 4. | Off types plants in seed parent at final inspection | 0.50 |

- Detasseling in female should be done before anthesis, as self pollination in female parent affects genetic purity of the hybrid seed. For effective hybrid seed production, silk in female should be ready earlier than anthesis in the male parent for proper pollination. Male parents should have longer duration of pollen shedding for continuous supply of pollen and the complete exertion of silk will help in higher seed setting. Wherever, there is problem of nicking, farmers can go for staggered planting or application of fertilizers (Urea, DAP) or growth regulators (GA, NAA, IAA) in the late parent.
- Timely weed management is essential for achieving the optimum yield and to overcome competition between crop and weeds for space, moisture and nutrients. The application of FYM @10-15 t/ha is desirable for root development, retention of moisture for long time, faster growth, and improved soil health and fertility. The fertilizer requirement depends on soil health, however, in general a dose of 120:60:40 kg of N:P:K per hectare is recommended. Soils deficient in zinc should be supplemented with 20-25 kg of $ZnSO_4$ per hectare. One third of entire N and full dose of P, K and $ZnSO_4$ are applied as basal dose during the field preparation. While two third of N dose is applied in equal amount at knee high stage and flowering stage of the crop. After first thinning (15-20 days to sowing), flowering and grain filling are the most critical stages of the crop for irrigation.
- Regular monitoring the seed production field is crucial. Monitoring should be done at (i) time of sowing: to monitor the land, isolation distance, planting ratio of male: female, proper sowing time, seed treatment; (ii) pre-flowering/vegetative stage: to verify the rouging and removal of off type plants; (iii) flowering stage: to check disease and pest infestation; (iv) post-flowering and pre-harvest stage: to remove the late and diseased plants; (v) harvesting time: to see the proper time of harvesting.

- Maize is highly sensitive to excess water and drought, thus schedule irrigation to provide adequate moisture to the plant at critical stages and prevent water logging.
- Timely weeding and intercultural operations are essential. Inter-cultivation is done manually and with inter-cultivators at a depth of 3 to 5 cm so that the roots are not damaged. Along with the last inter-cultivation operation earthing up should also be done. Weed control is particularly important in the production of hybrid maize seed, as many inbred lines fail to compete effectively with broad-level weeds. Pre-emergence soil application of Atrazine) at 1.5 kg per hectare in 1000 litres of water is recommended. There should be no weeds at the time of weedicide application. After application of weedicide soil should not be disturbed for four to five weeks.
- Ideally seed production plots should not have been planted to maize in the preceding season to prevent contamination by volunteer plants remaining in the field. In addition, all undesirable off type plants showing non-standard morphological characteristics (e.g. plant height; leaf shape and size; flowering habit; silk and ear characteristics; kernel shape size and colour) should be removed from the field. Two field inspections is undertaken, one prior to flowering and one during flowering. Open pollinated varieties have broad genetic base, and are phenotypically uniform for important economic characters. Roguing for off-types and malformed plants should be completed before pollen shedding and diseased plants affected by stalk rot should be rouged out from time to time. At harvesting sort out the off textured or off-coloured ears.
- Timely control of disease and insect pest is required for quality seed production. Following are the control measures of the major disease- and insects-pests.
  - *(i) Downy mildew:* Seed treatment with Metalaxyl W.P. @ 2-2.5 g/kg of seed and foliar spray of Mancozeb @ 2.5 g/litre or Metalaxyl MZ @ 2.0 g/litre provides excellent control over the disease.
  - *(ii) Turcicum leaf blight:* Two to four applications of Maneb or Zineb @ 2.5-4.0 gm/litre of water at 7-10 days interval provides good control of the disease.
  - *(iii) Maydis leaf blight:* Application of 2-4 sprays of Dithane M-45 or Zineb @ 2.0-2.5 gm/litre of water at 7-10 days interval from the first appearance of disease controls the spread of pathogen.
  - *(iv) Polysora rust:* Spray of Dithane M-45 @ 2-2.5 gm/litre at the beginning of appearance of symptoms provides good control of pathogen.
  - *(v) Post flowering stalk rot:* Avoidance of water stress at flowering stage and crop rotation also reduces the incidence of the disease. Further, application of bio-control agent such as *Trichoderma* formulation in furrow @ 10g/kg of FYM at 10 days prior to sowing provides good control.

(vi) *Banded leaf and sheath blight:* Removing lower 2-3 leaves, application of *Pseudomonas fluorescens* culture @16g/kg of seeds (as seed treatment) or 7g/litre of water for soil application coupled with foliar spray of Sheethmar or Validamycin @ 2.5-3.0 ml/litre of water provides reasonable control on the spread of the disease.

(vii) *Stem borer:* Spray of Chlorpyriphos @ 1-1.5ml/litre of water at 10-12 days after germination provides good control. The insecticide should be mixed in 800-1000 litre of water and evenly sprayed over the canopy per hectare. Additional 1-2 sprays after 7-10 days intervals further restricts the insect infestation. Alternatively, application of Carbofuron G 3% @ 0.6 kg a.i./ ha in the leaf whorls after 15-20 days of germination, provides protection against stem borer.

(viii) *Pink borer:* The control measure is similar to stem borer as mentioned above.

(ix) *Shoot fly:* Seed treatment with Imidacloprid @ 6ml/kg of seeds provides good control of the shoot fly. Early sowing during first fortnight of February avoids build up of shoot fly population.

- To avoid contamination, male parental lines should be harvested before female lines. To maintain the purity, diseased and pest infested cobs should be removed before shelling. The female cobs should be dried up to 13-14% moisture content before shelling. Shelling can be done manually or by power operated maize sheller. All under size, broken, damaged etc seeds should be removed for maintaining the high seed quality. Seed drying should be done till the seed moisture content reduces to 8%.

**Seed packing**

Maize seeds are packed into cloth, jute or multi-wall paper bags. Seed may be packed by hand (common in the case of small-scale seed producers) or using semi-automatic or automatic equipment (common in the case of medium and large-scale seed producers). A specified amount of seed is weighed out, the bag is hung and filled, the bag is sewn shut or heat sealed, a tag is attached, and the bag is coded. The code (which appears on the tag) generally includes information about the name of the OPV or hybrid, the seed lot number, the physical and genetic purity, seed treatment chemicals, date on which a germination test was performed and validity period.

**Seed storage**

Maize seed may be stored in bulk or bag. Seed conditioning facilities generally possess some bulk storage capacity, but it often proves inadequate when large quantities of seed must be stored for extended periods. Thus, seed is often bagged before being put into storage. Bagged seed has the advantage of being able to be transported and stacked. Recommended temperatures are as follows:

1. Short term (few months to a year) : $20^0$C/ 60% RH

2. Medium term (a year to 2-3 year) : $10^0$C/ 40% RH
3. Long term storage (>5 years) : $5^0$C/ 30% RH

**Seed Standards:**

| S. No. | Factor | Standards of each class | |
|---|---|---|---|
| | | **Foundation** | **Certified** |
| 1. | Pure Seed (Minimum) (%) | 98.0 | 98.0 |
| 2. | Inert Matter (Maximum) (%) | 2.0 | 2.0 |
| 3. | Other Crop seeds (Maximum) (per kg) | 5 | 10 |
| 4. | Other distinguishable varieties (Maximum) (per kg) | 10 | 20 |
| 5. | Weed Seeds (Maximum) (per kg) | None | None |
| 6. | Germination (Minimum) (%) | 90 | 90 |
| 7. | Moisture (Maximum) (%) | 12.0 | 12.0 |
| 8. | For vapour proof containers (Maximum) (%) | 8.0 | 8.0 |

# PRINCIPLES AND PRACTICES OF SEED PRODUCTION IN SORGHUM AND MILLETS

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## I. SORGHUM

Sorghum is common millet of India with wider utility. It is used a feed, food and raw material for agri-based industry. Botanically it is known as *Sorghum bicolor* L. and belongs to the family poaceae. It is an often cross pollinated crop, insects and wind are the pollinating agents. Sorghum is one of the main staple food for the world's poorest and most food insecure people across the semi-arid tropics. Globally, sorghum is cultivated on 41 million hectares to produce 64.20 million tonnes, with productivity around 1.60 tonnes per hectare. With exceptions in some regions, it is mainly produced and consumed by poor peasant farmers. India contributes about 16% of the world's sorghum production. It is the fourth most important cereal crop in the country. In India, this crop was one of the major cereal staple during 1950's and occupied an area of more than 18 million ha but has come down to 7.53 million ha. In India, sorghum is the most important cereal crop grown across semi-arid zones. The development and release of sorghum hybrids in India since 1963 has resulted in quantum jump in productivity from 570 kg /ha in 1970's to >1000 kg/ ha in recent years. The best season for seed production is November – December. The pollination should not coincide with rains. Rains prevent quality and effective seed setting. The temperature of 37$^{o}$c is favourable for seed setting.

### Floral biology

The sorghum panicles may be 4–25 cm long and 2–20 cm wide, which may be short and compact or loose and open. The inflorescence is a raceme consisting of one or several spikelets. Racemes vary in length depending upon number of nodes and length of internodes. The sessile spikelet varies in shape from lanceolate to almost round and ovate and is sometimes depressed in the middle. The lower glume is usually somewhat flattened and conforms more or less to the shape of the spikelet, while the upper one is more convex or boat-shaped. The seed may be enclosed by the glume or may protrude from the glume. The seed may be just visible or almost completely exposed. In sessile spikelets, there are two lemmas, two lodicules and a palea. The pedicelled spikelets are much narrower than the sessile spikelets and are usually lanceolate. They are male or neutral in sex, but may rarely have a rudimentary ovary. The lemmas are much reduced in size and only rarely does the upper lemma have awn. Sorghum has 2 pistils and 3 stamens. Each fluffy stigma is

attached to a short and stout style extending to the ovary. The anthers are attached to long thread like filaments.

**Anthesis and pollination**

The floral initiation (Primordial formation) starts at 30 to 40 days after germination (but may range from 19 to 70 days or more) Floral initiation marks the end of the vegetative growth and the meristematic activity. Sorghum usually flowers in 55 to 70 days in warm climates, but it could be as early as 30 days or as late as 100 days or more. The flowering (anthesis) in a panicle starts from the top and it travels successively to lower whorls. Flowering is completed over a period of 4 to 5 days (6-8 days under cooler conditions). Pollen is usually available for a period of 10 to 15 days because the heads in a field do not flower at the same time. Sorghum is predominantly a self-fertilized crop, but the cross pollination may occur to an extent of 2 to 10 percent. At flowering, the glumes open and the three anthers fall free, while the two stigmas protrude, each on a stiff style. Flowering occurs just before or just after sunrise, but may be delayed on cloudy damp mornings. The anthers dehisce when they are dry. For selfing, the panicle is merely covered with a paper bag. Pollen is shed freely and can always be collected in bags enclosing the spikes. Crossing made before 10.00 AM generally sets maximum amount of seed per head.

**Sorghum seed multiplication chain**

In India, the seed multiplication is in three stage generation system along with Nucleus seed (NS).

- Breeder seed (BS)
- Foundation seed (FS)
- Certified seed (CS)

The seed certification under law is voluntary as per Seeds Act of 1966 and is applicable to only notified kind of varieties. However, as per Draft Seeds Bill, 2002, only registered kind of varieties that prove DUS and VCU are eligible to be permitted under seed trade and voluntary certification system. Apart from SC, the seed is also sold as truthfully labeled seed (TLC). However the certified seed has the advantage of the seed crop being monitored by an authorized agency to ensure high genetic and physical purity, freedom from disease and pest, high germination and seedling vigor. The seed of different classes is produced based on demand forecasting of annual certified seed requirement depending on seed multiplication ratio, seed replacement rate and additional seed requirement.

**a) Nucleus seed**

The handful of initial seed obtained from selected individual plants of a particular variety produced by the originating breeder or the institute constitutes the nucleus seed. It is not covered under the purview of certification, is produced in small quantities on experiment stations by the

breeder under his direct supervision, and forms the basis for further multiplication of breeder, foundation, and certified seed.

**b) Breeder seed**

It is the progeny of nucleus seed. It bears a golden yellow tag issued by the producing breeder. Its production is organized by the ICAR through the ICAR institutes, Agricultural Universities and seed corporations in certain crops on the basis of indents received from Department of Agriculture, Ministry of Agriculture, Government of India. Breeder seed production also does not come under the purview of certification. However, production and genetic purity are monitored by the monitoring team consisting of the project coordinator, nominees of national seed corporation, state seed corporation and state seed certification agency. For the hybrid seed production chain, needs three basic seed lines: male sterile line (A- line), maintainer line (B- line) and restorer line (R- line). The seed of A- line is produced by planting A- and B- lines side by side in isolated. This process will give the A- line seed perpetually. The B- and R- lines can be reproduced easily like any other self pollinated variety. The seed obtained from breeder is normally in small quantities (up to few kilograms). Breeder seed plots should be at least 400 m away from other sorghum field, Johnson grass, and other forage or grassy sorghum types.

**c) Foundation seed**

It is the progeny of breeder seed. It is genetically and physically pure, satisfying the minimum certification standards. Foundation seed is produced by National Seed Corporation, State Seed Corporations, State Farms Corporation of India, State Agricultural Universities and designated public and private sector agencies. The foundation seed shall be the source for production of certified seed. Foundation seed bears white tag on certification. The breeder seed alone should be used to plant the foundation seed plots isolated from other sorghum fields at least by 300 m. Before flowering, grower should search for and destroy all volunteer plants on field borders and ditches, and in nearby fields lying within the isolation distance. Obvious off-types are roughed out before flowering to meet foundation seed standards. The off-types permitted at the final inspection depend on the seed certification standards and normally genetic purity should exceed 95%.

**d) Certified seed**

It is the progeny of foundation seed. It should satisfy the prescribed seed standards. In case of sorghum, Maharashtra State Seed Corporation (MSSC), Andhra Pradesh State Seed Development Corporation (APSSDC) and Karnataka State Seed Corporation (KSSC) are the four major agencies that produce certified seed. Various private seed companies compliment these public sector agencies in sorghum hybrid seed production. Some private companies market both private and public sector hybrids. To produce hybrids, the foundation seed of A- and R- lines are used by certified seed growers. This seed is normally certified by a team of specialists from the State seed certification agency,

scientists of research station and state seed corporation. Certified seed bears blue tag. Approved foundation seed alone should be used and planted in large contiguous blocks with an isolation of at least 200 m from other sorghum fields. Rouging can be practiced to remove off-types. The permitted off-types at the final inspection depend on the seed certification standards; normally off-types should not exceed 0.1%.

**Sorghum hybrid seed production**

The cytoplasmic-genetic male sterility, induced by interaction of sterility-inducing factors in the cytoplasm with the genetic factors contained in the nucleus, forms the basis of hybrid seed production. The sorghum hybrids can be developed as follows.

**a) Identification of potential hybrid parents (A-, B-, and R- lines)**

Potential male and female parents for hybrid seed production are identified by crossing male-fertile plants (inbreds, varieties, germplasm, breeding stocks in advanced generations, etc.) to a male-sterile line (A-line) and evaluating the corresponding hybrids in small plots in an observation nursery. A few plants of each cross are subjected to a bagging test, i.e., covering few panicles with paper bags before anthesis, and observing seed set under the bag after few weeks. A normal bisexual fertile panicle would exhibit nearly 100% seed set whereas in crosses with A-lines, the following three categories are encountered.

1. Hybrids without seed set (i.e., male-sterility) is maintained in these hybrids. The corresponding pollen parent is classified as a non-restorer / maintainer or B- line.
2. Hybrids with complete seed set under the bag (i.e., male fertility) being completely restored in these hybrids. The corresponding pollen parent is classified as a potential male parent / restorer line (R- line) and could be useful in producing hybrids.
3. Hybrids exhibiting partial seed set under the bag. Such hybrids and their male parents are rejected for further studies because experience shows that it is difficult to extract stable R-lines or B-lines from such parents.

The hybrids, with complete seed set under bag are evaluated visually local genotypes as checks. Various agronomic traits such as days to maturity, plant height, fodder yield, grain color, quality, panicle size, hybrid vigor, grain yield, threshability and resistance to diseases and pests are considered. Selected hybrids are advanced for further studies and their corresponding male parents are included in the R- line collection.

**b) Hybrid (A x R) seed production (certified seed)**

The hybrid seed is produced as certified seed under the vigilance of state seed certification agencies on a very large scale by private agencies, seed farms, experienced growers, and other extension organizations. Sorghum hybrids involve A × R seed production and are carried out according to the prescribed

standards of production and processing in terms of isolation distance, genetic purity, and seed quality.

Seed certification and seed law enforcement agencies have an important role to play in certified hybrid seed production and distribution, because the crop performance is dependent on the quality of the certified seed used. Although production of hybrid seed can be carried out by small individual growers, it is convenient to grow it in large compact blocks of 100-150 ha in a single or cluster of adjoining villages to avoid isolation problems. The quantity of certified seed produced depends upon the projected demand for the seed of a particular cultivar; normally an excess of 20% over the demand is produced. The requirements of isolation distance must be satisfied by a negotiated contract between a contract grower or group of farmers living in a community (seed village) and the seed firm. The seed companies train supervisory staff who will in turn advise and assist the seed growers in hybrid seed production across critical crop growth stages. Close communication between seed growers and the company supervisory staff during the entire hybrid seed production season facilitates quality hybrid seed production.

## II. PEARL MILLET

Pearl millet originated in tropical western Africa some 4000 years ago. The greatest numbers of both wild and cultivated forms of this species occur in this region. Pearl millet can grow in a wide range of ecological conditions and yield reasonably well even under unfavorable conditions of drought stress and high temperatures. It is mostly grown in countries with hot and dry weather, quite characteristic of the arid and semi-arid environments. Pearl millet grows on soils that are too sandy and light textured, too arid, too dry and too infertile for other cereals. It played a significant role in food security and preferred by resource poor farmers of dry regions for its better nutritional value.

### Botany

Seed production procedure in crop species depends on its mode of pollination which corresponds to its floral biology. The salient features of floral biology and mode of pollination in pearl millet are given briefly as under:

Pearl millet inflorescence is a compound terminal spike or panicle. It consists of a central rachis. It bears fascicles on rachillae, arranged in a spiral form. Each fascicle contains 1-2 spikelets surrounded by a whorl of bristles (*i.e.* involucre). A spikelet consists of two glumes and two florets. The lower floret is staminate and the upper hermaphrodite. The ovary is free and exposed and monocarpellary. The styles are free or adnate at base and bifid, terminating in brust-like stigmas. There are three stamens with penicillate and versatile anthers.

Pearl millet is a protogynous species. The styles start protruding two to three days after the emergence of the panicle. The stylar branches protrude first from the florets in the upper middle region of the panicle and then proceed both upwards and downwards. In the hermaphrodite flowers, the stigmas emerge faster than the anthers and hence stigmas receive pollen from inflorescence of other plants. The time required for complete stigma emergence varies from 2 to 3 days, depending on the environmental conditions. The two stigmas separate and diverge only after complete exertion of the styles. They remain fresh and receptive for two to three days, depending upon environment. The sequence of flowering practically excludes self-pollination in the same inflorescence, but it may occur between the inflorescences of the same plant.

By the time anther emergence commences, all stigmas will have emerged, and been pollinated, which avoids selfing under open-pollination conditions. The emergence of the first anther usually begins about three to four days after the first stigma has emerged. Protogyny and the time lag between stigma emergence and anther dehiscence favours cross-pollination, but asynchronous flowering of tillers prevents its full realization. The protogyny in the pearl millet is exploited for controlled cross pollination without resorting to emasculation. The inflorescence to be used as a female or male is covered with the glassine paper bag before any stigma is visible. Generally the safest stage is when about one third of the inflorescence is out of the flag leaf sheath. When all stigmas have emerged, the panicle can be considered ready for cross pollination. If selfed seed of the male parent is not required, pollen from it can be collected by bagging even those inflorescences in which stigmas have completely emerged. Fresh pollen from dehiscing anthers, visible as yellow powder in the transparent selfing bags, is collected by tapping the bagged inflorescence. The pollination is carried out by quickly removing the bag from the female inflorescence, dusting the pollen collected from the male inflorescence, and then re-bagging the pollinated inflorescence again.

**Seed production**

The best season for seed production is October - December. The temperature favourable for seed setting is 37°C. The pollination should not coincide with rains. Rain affects effective seed setting and production of quality seeds.

**A) Nucleus Seed Production**

The salient features of the procedure for the production of nucleus seed of OPVs, male sterile line, maintainer line and restorer line in pearl millet are described below:

**1. Open –pollinated variety**

***Season I – Isolation Block***

- Base seed of open pollinated variety is grown in an area of 0.1 to 0.2 ha maintaining strict isolation of at least 1000 m from any other plot of

pearl millet or wild pearl millet. One third of the commercial plant population should be maintained keeping at least 3000 - 5000 plants.

- Carefully observe at critical stages (tillering, pre-flowering, flowering, dough stage and maturity) and select 500 to 1000 plants with characters identical / typical to the released variety. Harvest and keep seed of each selected plant separately. Evaluate for seed characters.
- Keep half of the seed of each plant progeny as remnant seed.

***Season II – Progeny evaluation***

- Plant unreplicated progeny rows along with check rows (grown from the basic bulk seed of OPV) after every 15-20 rows.
- Compare progeny rows at critical stages and select 30 to 50% progenies confirming to the varietal characters.
- Bulk the remnant seed of selected progenies.

***Season III – Nucleus seed nursery***

- From the bulked remnant seed grow nucleus seed nursery in isolation (1000 m).
- The harvested seed is bulked and can be divided into five-six lots and kept under cold storage.
- One of these lots can be used as base seed for nucleus seed production when required and rest may be used for breeder seed production in subsequent years.

**2. Hybrids**

The nucleus seed production of hybrids essentially involves the seed production of their parental lines. In case of pearl millet, especially single cross hybrids are in vogue, thus an account of production of nucleus seed of single cross hybrids i.e. A line (Male Sterile), B line (Maintainer) and R line (Restorer) is given below:

**(i) Maintainer line ('B' lines):**

***Season I***

- Grow large number of plants of B line (0.05 ha.) and select and self about 1000 plants at the time of flowering.
- Finally select about 200 selfed plants confirming to the characters of maintainer line.

***Season II***

- Grow plant to row progeny of selfed plants in two replications retaining remnant seed.
- The progeny rows are studied for the diagnostic characters and rows not confirming to the characteristics of the line are rejected.
- Identify the best progeny rows (25-30%).
- Bulk the remnant seed of selected best lines.

***Season III***

- Grow the bulk seed of remnant seed in isolation.

- Bulk the seed of all the plants after the harvest.
- This forms the nucleus seed bulk of B line.

**(ii) MS A line ('A' lines):**

***Season IV***

- Grow A and B lines in alternate rows. Seeds of B line will be those obtained from rejuvenation as given in the maintenance of B lines.
- Make 200 – 250 paired crosses between A and B plants.
- Care is taken to cross A and B plants confirming to the line standards only.
- Paired crosses among A and B lines should be labelled *viz.* A1 × B1, A2 × B2 etc. and harvested seed of each pair should be kept separately.

***Season V***

- Grow the pairs, respective A line (crossed seed) and B line (selfed seed) in alternate rows.
- Retain a portion of seed as remnant seed.
- Observe critically pairs of A and B lines for all the characters including height, flowering and typical morphological characters.
- Observe for pollen shedders in A lines. The A line progenies showing pollen shedders and corresponding B lines should also be rejected.
- Identify uniform pairs of A and B lines which confirm to the standards of parental lines.
- Remnant seed of the A lines of the selected pure pairs is bulked. This forms nucleus seed bulk of A line.

**(iii) Restorer line ('R' lines):**

***Season I***

- Grow large number of plants of R line (0.05 ha.). Self a number of plants (about 1000) confirming to the standards of line at the time of flowering.
- Finally select about 200 selfed plants based on field studies as well as observation in laboratory for seed colour, shape etc.

***Season II***

- Grow plant to row progeny of selfed plants in two replications.
- Retain a portion of selfed seed of each plant as remnant seed.
- The progeny rows are studied for the qualitative (diagnostic) characters. The lines not confirming to the characteristics of the line are rejected.
- Evaluate the lines for yield and agronomic score for other economic characters.
- Identify the best progeny rows (30 to 50%) based on all characteristics mentioned above. In the progeny row testing if adequate number of progeny rows confirming to the line standards are not obtained, selfing for one or more generations will be required. These selfed plants of R line should also be tested for their restoration ability.
- Bulk the remnant seed of best lines.

***Season III***

- Grow the bulk seed of remnant seed in isolation.
- Bulk the seed of all the plants after the harvest.
- This forms the nucleus seed bulk of R line.

**B. Breeder/ Foundation seed production**

Breeder seed is produced from nucleus seed stock while foundation seed is produced from breeder seed as per detail is given in seed class. The minimum isolation distance is 1000 m for breeder/ foundation seed of A, B, R lines and OPVs.

***1. Male Sterile (A) line and maintainer (B) line***

- Multiply 'A' lines by planting A and B lines in alternate set usually in the ratio of 4:2.
- Plant 4-8 border rows of B line around production block to insure adequate pollen supplied to A line.
- Careful and strict rouging is a necessity in A and B lines. Rogue out pollen shedders in the A lines, if any.
- Harvest B-line rows immediately after completion of flowering period.
- Carefully harvest the A line rows and bulk the seed.
- Separate breeder seed production plot should be raised under isolation for nucleus seed stock as base material.
- For breeder seed production of B lines raise separate production plot of 0.1 ha area under isolation (1000 m) as bulk planting from nucleus seed stock.
- Follow rouging and other procedures as detailed in nucleus seed production method.

***2. Restorer (R) line and OPV***

- Breeder / foundation seed production of R lines and OPVs is done by bulk planting under isolation from the nucleus seed stock.
- Seed plots should be 0.1 – 0.2 ha for R lines/ OPVs and at least 3000 – 5000 plants should be maintained.
- Select 500 – 1000 panicles (depending on the requirement) from the bulk planting.
- Harvest and bulk the seed.
- After harvest, the seed be dried, processed and stored in safe containers.

**C. Certified seed**

Certified seed of OPV and hybrid is produced in pearl millet. The stipulated isolation distance for certified seed production, both for hybrids and OPVs is 200 m. The pattern of planting and production of OPVs is the same as for the breeder and foundation seed production. Production must be acceptable to seed certifying agency and fulfill all requirements of certification.

Certified seed is generally arranged through contract growers. Some private seed companies also undertake certified seed production programmes. The certified seed of a hybrid is produced by growing male sterile line with a specified restorers line in an isolated field.

Synchronisation of A and R line is crucial for certified hybrid seed production. This can be manipulated by (1) differential dates of sowing (2) manipulating of water and fertilizer to one of the hybrid parents and (3) removal of extra early tillers in A or R line to synchronise the ability of pollen shedding and stigma receptivity.

Off-type plants in R line and pollen shedders in A line should be roughed out carefully to maintain genetic purity. A satisfactory certified seed production can be achieved if seed village concept is followed.

**Field standards**

Bajra is a highly cross-pollinated crop with 80% of cross-pollination. The crop should be raised in isolation and seeds should be allowed to set by open-pollination. The isolation distance maintained between the varieties is 400 metres for foundation seed and 200 metres for certified seed production.

**Seed standards**

The percentage of minimum physical purity of certified and foundation seeds should be 98% with a minimum of 80% of germination capacity and 5 - 12% of moisture content. The presence of inert matter should not exceed 2.0%.

## III. FINGER MILLET

Among millet crops, finger millet figures prominently; it ranks fourth in importance after sorghum, pearl millet and foxtail millet. Finger millet cultivation is more widespread in terms of its geographical adaptation compared to other millets. It has the ability to withstand varied conditions of heat, drought, humidity and tropical weather. It is an important staple in many parts of eastern and southern Africa, as well as in South Asia. The crop is productive in a wide range of environments and growing conditions, from southern Karnataka state in India to the foothills of the Himalayas in Nepal, and throughout the middle-elevation areas of Eastern and Southern Africa.

**Morphological description**

Finger millet is a tufted annual crop, growing to a height of 30–150 cm and maturing in 75–160 days. Leaves are narrow, grass-like and capable of producing many tillers and nodal branches. The panicle consists of a group of digitally arranged spikes often referred to as fingers. The spikelets are made up of 4–10 florets arranged serially on the finger. All florets are perfect flowers with the exception of the terminal ones which may sometimes be infertile. The grain

is oblong to round and oval, reddish brown in colour with the grains' surface finely corrugated. Typically a tropical, rainfed crop, it is one of the best suited for dry farming. Finger millet is very adaptable and thrives at higher elevations than most other tropical cereals.

**Flowering and anthesis**

Complete emergence of inflorescence in finger millet required about 10 days and flowering attains 7-8 days. The flower open between 1 to 5 AM and progress from top to bottom in a finger, however, in a spikelet the order is reversed and proceeds from bottom to top and bigger to smaller flower. The stigma is receptive for a very short period after its emergence from the glumes. The period of anthesis being very short, is conducive for self-pollination and cross pollination is very rare.

**Seed production**

The best season for seed production is December – January. Pollination should not coincide with rains for quality and effective seed setting. The temperature of 37°c is favourable for seed setting.

**Field standards**

Ragi is a self-pollinated crop and should be raised in isolation. The isolation distance maintained between the varieties is 3 metres for both foundation and certified seed production to maintain the varietal purity.

**Seed standards**

The percentage of minimum physical purity of certified and foundation seeds should be 97% with a minimum of 75% of germination capacity and 12% of moisture content. The presence of inert matter should not exceed 2.0%.

**Field inspection**

A minimum of two inspections should be done between flowering and maturity stages by the Seed Certification Officer. The first inspection is done at the time of flowering to check the isolation and off-types and the second done during the maturity stage prior to harvest to check the off-types and to estimate the yield.

**Harvesting and processing**

Harvest is done once the earheads are physiologically mature. Physiologically mature earheads will turn from brown to green colour. Harvesting is done in two pickings since, the maturation of the earheads are not uniform because of the tillering habit of the crop. Second harvesting should be done seven days after the first one. Mature earheads should be harvested and threshed with bamboo sticks. Threshed grains are further cleaned by winnowing.

**Drying and storage**

The cleaned seeds should be sun dried to attain a safe moisture level of 12%. Care should be taken while drying to avoid mechanical injury to the seeds and contamination. Seeds can be stored upto 13 months under proper storage conditions.

## IV. KODO MILLET

Kodo millet is a native of India and is in cultivation since time immemorial. Greater diversity of *Paspalum* species in the Hindustan centre was observed. The crop was domesticated in southern Rajasthan and Maharashtra some 3000 years ago. The crop is known to be spread throughout the tropical regions of the world. Kodo millet (*Paspalum scrobiculatum*) is grown throughout Asia and Africa, but is only cultivated as a minor grain crop in India, where it is of great importance in the Deccan Plateau. Its cultivation as grain crop in India is generally confined to Gujarat, Karnataka, and parts of Tamil Nadu. In other parts of the world it is grown as a forage crop.

**Botany**

Inflorescence is a spike or spike like racemes. Each spikelet consists of 1 or 2 flowers and bears at the base bracts or glumes, one placed a little above and opposite the other. These two are empty while a third one called lemma is flowering i.e. it enclosed a flower in its axil. Opposite the flowering glume or Lemma, there is somewhat smaller, two nerved glumes called Palea. Spikes 2-6, sessile usually distant and spreading, rachis herbaceous, broad with ciliate margins. Spikelets usually 2 ranked, 2-3 mm diameter, sessile or shortly pedicilate, broadly elliptic or suborbicular imbricate. Androecium: Stamens 3, filamentous, anthers 3, 2 locules, open by longitudinal sutures, versatile and pendulous. Gynoecium: Monocarpellary, ovary superior, one cell with one ovule, stigma 2, feathery, style distinct. Grain: Utricle type in which pericarp is like a sac usually attached to endosperm at only one point. Grains are rotundate-elliptic, convex in front, flat on back of palea, scutellum up to half the length of the grain.

**Flowering behaviour**

Flowers of kodo millet are cleistogamous in nature and thus remained closed. Protogynous flowers occurs rarely in few genotypes. The opening of the flowers occurs between 7.30 to 8.00 AM in Nagpur conditions. Only 5% flowers open and remaining being cleistogamous. The glumes begin to open at 2.30 AM. The anthers become visible through opening at 2.40 AM, emerge at 3.15 AM and comes completely out at 3.30 AM. Anthers dehisce at 3.35 AM and glumes close completely at 3.45 AM. The dehiscence of anther occurs by a slit at one end and speed up gradually. Mostly the dehiscence of anther begins from the middle and

proceed to both ends. The feathery stigma dry up in the evening. The anthers remains fresh and do not wither till next morning. The lodicules are fleshy and do not shrink immediately after the anthesis of flowers. They remain fleshy for 6 to 8 hour after opening the glume and then dried up. The grains mature in 30¬40 days after flowering and remain tightly enclosed by the hardened fourth glume and its palea and have various shades of brown colour.
The best time of anthesis is between 5.45 and 7.30 AM. In this period a single floret of panicle is open for 20 to 30 minutes. The stigma comes first during anthesis of flowers and anthers arise just after the emergence of stigma.

**Seed production**
Seed production can be done in June – July and February – March. The pollination should not coincide with rains for quality and effective seed setting.

**Field standards**
Kodo millet is a self-pollinated crop. The crop should be raised in isolation. The isolation distance maintained between the varieties is 3 metres for both foundation and certified seed production to maintain the varietal purity.

**Seed standards**
The percentage of minimum physical purity of certified and foundation seeds should be 97% with a minimum of 75% of germination capacity and 12% of moisture content. The presence of inert matter should not exceed 2.0%.

**Seed selection and sowing**
Seeds used for seed production should be of good quality certified seeds from an authenticsource. Seeds should be healthy with required germination percentage. In North India, sowing should be done in mid June to mid July and in South India during September – December. Recommended seed rate is 4 kg/acre (10 kg/ha). Selected seeds should be treated with *Azospirillum* @ 60 gms/kg of seeds. Treated seeds should be sown with a spacing of 30 x 10 cm. Seeds should be sown at the depth of 3 – 4 cm.

**Roguing**
Roguing should be done often to remove the off types, volunteer plants and diseased plants from the seed production field to avoid the genetic contamination. Roguing should be done up to the flowering stage. Maximum percentage of off type permitted at the final inspection is 0.05% for foundation and 0.10% for certified seed production.

**Field inspection**
A minimum of two inspections should be done between flowering and maturity stages by the Seed Certification Officer. The first inspection is done at the time

of flowering to check the isolation and off-types and the second done during the maturity stage prior to harvest to check the off-types and to estimate the yield.

**Harvesting and processing**

Harvest is done once the earheads are physiologically mature. Normally crop is ready for harvest in 100 days. Physiologically mature earheads will turn from brown to green colour. Plants are cut close to the ground level, bundled and stacked for a week before threshing. The earheads are threshed by trampling under the feet of bullocks. The threshed grains are further cleaned by winnowing.

**Drying and storage**

The cleaned seeds should be sun dried to attain a safe moisture level of 12%. Care should be taken while drying to avoid mechanical injury to the seeds and contamination. Seeds can be stored up to 13 months under proper storage conditions.

## V. FOXTAIL MILLET

Foxtail millet (*Setaria italica*) is also known as Italian or German-Hungarian or Siberian millet. Foxtail millet was considered to be domesticated in the highlands of central China. The main production regions of the world include China, parts of India, Afghanistan, Central Asia, Manchuria, Korea, and Georgia. It is also one of the specialty crops in Japan. In Asia it is mainly grown for human consumption. It is cultivated in both tropical and temperate regions. The crop can be grown successfully in areas receiving 750 mm of annual rainfall. Foxtail millet is essentially a grain crop of about 100 days duration suited to conditions of low and moderate rainfall ranging from 500 to 700 mm. It can be grown in higher altitudes (up to 1830 m above MSL) and is an important food grain in the foothills of Himalayas.

It is a crop grown almost throughout the year in different parts of the country. Cultivation of foxtail millet in the lower Deccan Plateau including high lands of Andhra Pradesh, Karnataka and Tamil Nadu account for about 90 per cent of the area in the country. In the hilly regions of North India, foxtail millet is sown with other kharif crops and matures in about 2 months, providing food during scarcity periods. In Punjab, Himachal Pradesh and U.P. it is grown from June-July to September-October either as a border or as a mixed crop with several kharif crops.

**Botany**

The inflorescence of foxtail millet has a main stalk with shortened side branches bearings spikes and bristles. The first flowers of foxtail millet may open when three fourths of the panicle emerges from the sheath, or as many as

five days after full emergence. Flowering proceeds from the top of the head downward in each of the panicle branches. A large head may take 8 to 16 days to compete flowering. A single floret may remain open about 30 min, and about 80 min are required for the complete blooming process, which is hastened by high temperatures and low humidity.

**Seed production**

Best season for seed production is June - July and February – March. The pollination should not coincide with rains for quality and effective seed setting.

**Field standards**

Foxtail millet is a self-pollinated crop and should be raised in isolation. The isolation distance maintained between the varieties is 3 metres for both foundation and certified seed production to maintain the varietal purity.

**Seed selection and sowing**

Seeds used for seed production should be of good quality certified seeds from an authentic source. Seeds should be healthy with required germination percentage. Recommended seed rate is 2 kg/acre (5 kg/ha). Selected seeds should be treated with *Azospirillum* @ 125 gms/kg of seeds. Treated seeds should be sown with a spacing of 30 x 10 cm at a depth of 3 – 4 cm.

**Roguing**

Roguing should be done often to remove the off types, volunteer plants and diseased plants from the seed production field to avoid the genetic contamination. Roguing should be done up to the flowering stage. Maximum percentage of off types permitted at the final inspection is 0.05% for foundation and 0.10% for certified seed production.

**Field inspection**

A minimum of two inspections should be done between flowering and maturity stages by the Seed Certification Officer. The first inspection is done at the time of flowering to check the isolation and off-types and the second inspection is done during the maturity stage prior to harvest to check the off-types and to estimate the yield.

**Harvesting and processing**

Harvest is done once the earheads are physiologically mature. Normally crop is ready for harvest in 80 - 100 days after sowing. Physiologically mature earheads will start to dry. Plants are either harvested intact with earheads or earheads alone. The earheads are dried before threshing. The earheads are threshed by stone roller or trampling under the feet of bullocks. The threshed grains are further cleaned by winnowing.

**Drying and storage**

The cleaned seeds should be dried under the sun to attain a safe moisture level of 12%. Care should be taken while drying to avoid mechanical injury to the seeds and contamination. Seeds can be stored up to 13 months under proper storage conditions.

**Seed standards**

The percentage of minimum physical purity of certified and foundation seeds should be 97% with a minimum of 75% of germination capacity and 12% of moisture content. The presence of inert matter should not exceed 2.0%.

## VI. PROSO MILLET

Proso millet *(Panicum miliaceum)* is also known as common millet. It is believed to have been domesticated in central and eastern Asia and, because of its ability to mature quickly, was often grown by nomads. This type of millet is of ancient cultivation and is known to be grown in China since 3000 BC. Proso millet is widely grown in northern China, Mongolia, Korea, south-eastern Russia, Afghanistan, Pakistan, India, and southern Europe. It was also introduced to North America. Proso millet is well adapted to many soil and climatic conditions. Being a short-season crop with a low water requirement, it grows further north (up to 54deg.N latitude) than the other millets and also adapts well to plateau conditions and high elevations. The proso plant is considered a short-day plant and usually an erect annual, 30 to 100 cm tall with few tillers and an adventitious root system.

**Botany**

Proso stems and leaves are covered with slight hairs. The leaves may be up to 30 cm long with a short ligule but no auricles. The stem is terminated by a drooping panicle 10 to 45 cm long that may be open or compact. Proso ($2n = 36$) is considered a self-pollinated crop, but natural cross-pollination may exceed by 10% .

**Anthesis and Pollination**

Flowers open between 10 AM and 12 noon, as the day temperature rises. The spikelets open and close within about seven minutes and hence self-pollination is predominant, though a very small amount of cross pollination cannot be ruled out. Anthesis is basipetallic.

**Seed production**

The common millet responds to good cultivation. It is sown as a cold weather crop in October-November in the states of Andhra Pradesh and Tamil Nadu, and is practically the last crop to be sown in the year. In Maharashtra, the crop

is transplanted in July. In North Bihar and Eastern Uttar Pradesh, it is sown in March utilizing receding moisture as well as residual fertility especially in fields vacated by potato, peas, mustard and wheat. Seed rate of 8 to 10 kg per hectare is either broadcast or sown through seed drills in rows about 22 cm apart and covered with a brush harrow. The crop is mostly rainfed, but in some parts of Tamil Nadu, it is grown under well irrigation. Moderately warm weather is necessary for germination of the seed and growth of the plant. Proso germinates well at temperatures of 10deg. to 45°C, but does not germinate at 5° or 50°C. The highest rate of germinations is between 35° and 40°C.

**Field standards**

Proso millet is a self-pollinated crop and should be raised in isolation. The isolation distance maintained between the varieties is 3 metres for both foundation and certified seed production to maintain the varietal purity.

**Seed standards**

The percentage of minimum physical purity of certified and foundation seeds should be 97% with a minimum of 75% of germination capacity and 12% of moisture content. The presence of inert matter should not exceed 2.0%.

**Seed selection and sowing**

Seeds used for seed production should be of good quality certified seeds from an authentic source. Seeds should be healthy with required germination percentage. Recommended seed rate is 4 kg/acre (10 kg/ha). Selected seeds should be treated with *Azospirillum* @ 60 gms/kg of seeds. Treated seeds should be sown with a spacing of 30 x 10 cm. Seeds should be sown in June – July onset of monsoon rains. Summer crop should be sown in the month of February – March. Seeds are broadcast manually or by seed driller in furrows at a depth of 3 – 4cm.

**Roguing**

Roguing should be done often to remove the off types, volunteer plants and diseased plants from the seed production field to avoid the genetic contamination. Roguing should be done up to the flowering stage. Maximum percentage of off types permitted at the final inspection is 0.05% for foundation and 0.10% for certified seed production.

**Field inspection**

A minimum of two inspections should be done between flowering and maturity stages by the Seed Certification Officer. The first inspection is done at the time of flowering to check the isolation and off-types and the second inspection is done during the maturity stage prior to harvest to check the off-types and to estimate the yield.

**Harvesting and processing**

Harvest is done once the earheads are physiologically mature. Normally crop is ready for harvest in 65 - 75 days after sowing. The crop should be harvested when two thirds of the seeds are ripe. The harvested earheads are threshed by hand or trampling under the feet of bullocks. The threshed grains are further cleaned by winnowing.

**Drying and storage**

The cleaned seeds should be sun dried to attain a safe moisture level of 12%. Seeds can be stored up to 13 months under proper storage conditions.

**VII. LITTLE MILLET**

The origin of this crop is not well documented except for the probable Indian origin since it is endemic to India and has a name in all vernacular languages of India. *Panicum miliare* or little millet is cultivated or naturalized throughout India and Sri Lanka, and cultivated in neighbouring countries. The distribution of the crop is limited to India and little diversity is found elsewhere. The luxuriant presence of *Panicum psilopodium* Trin., a wild relative of little millet in India, is suggestive of Indian origin. The crop can grow well in drought conditions and considered as a good famine food as it can produce some grain even under severe drought conditions when all the other crops fail to produce. It is a typical dryland crop suitable for the areas with low rainfall and poor soils. Little millet is able to thrive on marginal soils which otherwise yield nothing, and mature as a crop even during famine years. It is a. hardy crop which ran withstand drought better than most of the other cereal crops and also water-logging to a certain degree. If the crop fails, the farmer stands to lose very little as the cost of production as well as the land value is negligible.

**Anthesis and Pollination:** The hermophrodite flowers which open in basipetallic pattern have brief and rapid anthesis period. The glumes open for not more than 2-3 minutes, and self-pollination is a rule. There is hardly any natural cross pollination. Under Indian conditions, the flowers open between 9 AM and 12 noon. Emasculation and artificial pollination is difficult in view of small flower size but not impossible. Encouraging results have been obtained by the contact method of crossing.

**Seed production**

Seed production can be done during June – July and February – March. The pollination should not coincide with rains for quality and effective seed setting.

**Field standards**

Little millet is a self-pollinated crop and should be raised in isolation. The isolation distance maintained between the varieties is 3 metres for both foundation and certified seed production to maintain the varietal purity.

**Seed standards**

The percentage of minimum physical purity of certified and foundation seeds should be 97% with a minimum of 75% of germination capacity and 12% of moisture content. The presence of inert matter should not exceed 2.0%

**Seed selection and sowing**

Seeds used for seed production should be of good quality certified seeds from an authentic source. Seeds should be healthy with required germination percentage. Recommended seed rate is 4 kg/acre (10 kg/ha). Selected seeds should be treated with *Azospirillum* @ 60 gms/kg of seeds. Treated seeds should be sown with a spacing of 30 x 10 cm. Seeds should be sown in June – July at the onset of monsoon rains. Summer crop should be sown in the month of February – March. Seeds are broadcast manually or by seed driller in furrows at a depth of 3 – 4 cm.

**Roguing**

Roguing should be done often to remove the off types, volunteer plants and diseased plants from the seed production field to avoid the genetic contamination. Roguing should be done up to the flowering stage. Maximum percentage of off types permitted at the final inspection is 0.05% for foundation and 0.10% for certified seed production.

**Field inspection**

A minimum of two inspections should be done between flowering and maturity stages by the Seed Certification Officer. The first inspection is done at the time of flowering to check the isolation and off-types and the second inspection is done during the maturity stage prior to harvest to check the off-types and to estimate the yield.

**Harvesting and processing**

Harvest is done once the earheads are physiologically mature. Normally crop is ready for harvest in 80 - 85 days after sowing. The crop should be harvested when two thirds of the seeds are ripe. The harvested earheads are threshed by hand or trampling under the feet of bullocks. The threshed grains are further cleaned by winnowing.

**Drying and storage**

The cleaned seeds should be sun dried to attain a safe moisture level of 12%. Seeds can be stored upto 13 months under proper storage conditions.

## VIII. BARNYARD MILLET

Barnyard millet belongs to genus *Echinochloa* of the family Poaceae. Different Echinlochloa species particularly *E. frumentacea* (L.), *E. colona* (L.), *E. crusgalli* (L.) Beauv. And *E. utilis* (Ohwi ex Yabuno) are cultivated. Barnyard millet is the fastest growing of all millets and produces a crop in six weeks. It is grown in India, Japan and China as a substitute for rice when the paddy crop fails. The plant has attracted some attention as a fodder in the United States and Japan.This millet is widely grown as a cereal in India, Pakistan, and Nepal and for fodder and as bird food elsewhere. This crop prefers lighter and drier soils well supplied with micro and macronutrients. Generally barnyard millet can be grown under different conditions in warmer regions but the highest yields (50 times higher than in poor growing conditions) are reached on good fertile soils, which shows its certain adaptability.

### Botany

The height of the plant varies between 50 and 100 cm. The inflorescence is 15cm long, densely branched, and usually has purple-ting with awnless scabrous spikelets. It can mature in less than 40 days, and yields about 700-800 kg grain, and 1000- 2000 kg of straw per hectare. It has coarse leaves and varies from one to five feet in height depending on available moisture and fertility. The seed - head is a compact panicle - type inflorescence four to eight inches long, purplish in colour, with awnless seed. Conversely, wild barnyard grass has seed w ith conspicuous awns and a more open - branched panicle.

### Seed production

The crop is able to evade drought by its quick maturity. Best season for seed production is September – October and February – March. The pollination should not coincide with rains for quality and effective seed setting.

### Field standards

Barnyard millet is a self-pollinated crop and should be raised in isolation. The isolation distance maintained between the varieties is 3 metres for both foundation and certified seed production to maintain the varietal purity. Land to be used for seed production of barnyard millet shall be free of volunteer plants.

### Seed standards

The percentage of minimum physical purity of certified and foundation seeds should be 97% with a minimum of 75% of germination capacity and 12% of moisture content. The presence of inert matter should not exceed 2.0%.

### Seed selection and sowing

Seeds used for seed production should be of good quality certified seeds from an authentic source. Seeds should be healthy with required germination percentage. Recommended seed rate is 4 kg/acre (10 kg/ha). Selected seeds should be treated with *Azospirillum* @ 60 gms/kg of seeds. Treated seeds should be sown with a spacing of 30 x 10 cm. Seeds should be sown in September - October at the onset of monsoon rains. Summer crop should be sown in the month of February – March. Seeds are broadcast manually or by seed driller in furrows at a depth of 3 – 4 cm.

**Roguing**

Roguing should be done often to remove the offtypes, volunteer plants and diseased plants from the seed production field to avoid the genetic contamination. Roguing should be completed within the flowering stage of the crop. Maximum percentage of off-types permitted at the final inspection is 0.05% for foundation and 0.10% for certified seed production.

**Field inspection**

A minimum of two inspections should be done between flowering and maturity stages by the Seed Certification Officer. The first inspection is done at the time of flowering to check the isolation and off-types and the second inspection is done during the maturity stage prior to harvest to check the off-types and to estimate the yield.

**Harvesting and processing**

Harvest is done once the earheads are physiologically mature. Normally crop is ready for harvest in 75 - 90 days after sowing. The crop should be harvested when two thirds of the seeds are ripe. The harvested earheads are threshed by hand or trampling under the feet of bullocks. The threshed grains are further cleaned by winnowing.

**Drying and storage**

The cleaned seeds should be sun dried to attain a safe moisture level of 12%. Seeds can be stored up to 13 months under proper storage conditions.

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# PRINCIPLES AND PRACTICES OF SEED PRODUCTION IN SOYBEAN

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Over the last 20-30 years, consistent improvements in average yield levels and reductions in production costs have steadily improved the competitive position of soybeans among arable crops. Among oilcrops, soybean covers a leading role at the global scale: today, soybeans account for about 35% of total harvested area devoted to annual and perennial oilcrops. The crop's share in global oilseed output is estimated at over 50%. Soybean cultivation is highly concentrated geographically, with only four countries - USA, Brazil, Argentina and China - accounting for almost 90% of world output. Asia - excluding China - and Africa, together account for only 5% of production. India and Bolivia are also significant producers of soybeans.

Soybean (*Glycine max* L.) is a potential oilseed of India. It accounts for nearly 50 percent of total production of oilseed crop in the world. Its seed, which contains about 40 percent protein and 20 percent oil, provides approximately 60 percent of the world supply of vegetable protein and 30 percent of the oil. Soybean protein is rich in the valuable amino acid lysine (5%), in which most of the cereals are deficient. In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin) and its sprouting grain contains a considerable amount of vitamin C. Vitamin A is present in the form of precursor carotene which is converted into vitamin A in the intestine. A large number of Indian and western dishes such as bread, chapatti, milk, sweets, pastries etc., can be prepared with soybean. It is used for manufacturing vanaspati ghee and other industrial production of different antibiotics.

Soybean builds up the soil fertility by fixing large amounts of atmospheric nitrogen through root nodules. It can be used as fodder, forage can be made into hay, silage etc. Its forage and cake are excellent nutritive foods for livestock and poultry. Soybean being the richest, cheapest and easiest source of best quality proteins and fat having a vast multiplicity of uses as food and industrial products is called as wonder crop. Soybean ranks third in oil provides vegetable economy in India, after groundnut and rapeseed-mustard It is one of the important crops of the world cultivated over an area of 103 million hectares with production of 263.5 million metric tones. The important soybean producing countries are U.S.A., China, Brazil and Mexico. In India it is cultivated in 9.55 M ha with production of 12.66 M tones and the productivity is 1325 kg /ha.

## Area of Cultivation

Production of soybean in India (4% of global) at the present time is restricted mainly to Madhya Pradesh, Uttar Pradesh, Maharashtra and Gujarat. It is also grown on a small acreage in Himachal Pradesh, Punjab and Delhi.

**Floral biology and pollination**

It has typical papillionaceous flower with a tabular calyx of five unequal sepal lobes and five- parted corolla consisting of posterior banner petal, two lateral wing petals and two anterior keel petals in contact with each other but not fused. The androecium consists of 10 stamens arranged in diadelphous manner. The single pistil is unicarpellate and has one of the four campylotropous ovules alternating along the posterior suture. The elevated stamens form a ring around stigma. The pollination often occurs before the opening of the flowers. The pollen is shed directly on stigma. Due to presence of cleistogamy, there is very high percentage of self-fertilization. The natural out-crossing is generally less than 1 per cent. The time from pollination to fertilization is 8-10 hours. The day of opening of flowers is likely the day of fertilization or one day after it.

**Season of growing soybean**

Soybean grows well in warm and moist climate. A temperature of 26.5 to 30°C appears to be the optimum for most of the varieties. Soil temperatures of 15.5°C or above favor rapid germination and vigorous seedling growth. A lower temperature tends to delay the flowering. Day length is the key factor in most of the soybean varieties as they are short day plants. In northern India soybean can be planted from third week of June to first fortnight of July.

**Method of seed production**

Soybean is a self-pollinated crop. To maintain the varietal purity in soybean, an isolation distance of 3 meters is maintained between varieties for certified and foundation seed production.

**Land selection**

The land selected should not be cultivated with the same crop in the previous season. The land should be fertile with a pH range of 6 – 7.5 and proper drainage facility. Land should be ploughed for 3 – 4 times. During last plough apply FYM @ 10 truckloads per acre (25 truckloads per hectare).

**Seed selection and sowing**

Certified seeds of good quality should be obtained from authenticated source. Seeds should be healthy with good germination percentage. Remove the off colour and out sized seeds. The seed rate for soybean is 16 kg/acre (40 kg/ha). Selected seeds should be treated with Trichoderma viride @ 10gm/kg of seeds to control seed borne diseases like root rot and wilt. Shade dry the seeds for 30

minutes before sowing. Seeds can also be treated with cow's urine, cow pat pit, panchagavyam etc. Seeds should be treated with Rhizobium @ 250 gms/acre to facilitate natural nitrogen fixation by plants. Soybean seeds are treated by wet sand method for uniform germination. For this, take equal quantity of seeds and sand. Mix water with sand @ 50 ml/kg and take a broad vessel and add seeds and sand in alternate layers and cover the vessel with a cloth. Remove the seeds after 16 hrs using a sieve. This seed treatment improves the germination capacity of the seeds. Treated seeds should be dibbled at 2 - 4 cm depth in seed beds. Sowing should be planned in such a way that the harvest does not coincide with rains. It will reduce the seed quality. The spacing is maintained at 30 x 10 cm for soya bean.

**Soybean crop rotation**

Mixed cropping of soybean with maize, mandua and sesamum has been found feasible and more remunerative. In mixed cropping of maize and soybean, plant maize at 100 cm row spacing keeping plant to plant distance 10 cm and three rows of soybean in between maize rows. A randomized complete block study on row spacing and plant density showed a higher protein content and lower oil content at 19 cm compared to 75 cm row spacing. Soybean has tremendous scope as an intercrop in arhar, cotton, and upland rice in northern India. In southern part of the country, soybean has a good scope as intercrop in sorghum, cotton, sugarcane, arhar and groundnut. In central India, soybean has been found very remunerative on the fallow lands in Kharif. Some of the common rotations followed in north India are: Soybean – wheat , Soybean – potato , Soybean – gram , Soybean – tobacco , Soybean – potato – wheat

**Cultivation**

It requires a good seedbed with a reasonable fine texture and not too many clods. Land should be well levelled and free from crop stubble. One deep ploughing with mould board plough followed by two harrowing or two ploughing with local plough are sufficient. There should be optimum moisture in the field at the time of sowing.

**Sowing of soybean**

The sowing should be done in lines 45 to 60 cm apart with the help of seed drill or behind the plough. Plant to plant distance should be 4-5 cm. The depth of sowing should not be more than 3-4 cm under optimum moisture conditions. Seed rate of soybean depends upon germination percentage, seed size and sowing time.

**Fertilizer and Nutrient Management**

For obtaining good yields of soybean apply 15-20 tonnes of farm yard manure or compost per hectare. Being a legume crop, soybean has the ability to supply

its own nitrogen needs provided they have been inoculated and there is efficient nodulation in the plant. An application of 20-30 kg nitrogen per hectare as a starter dose will be sufficient to meet the nitrogen requirement of the crop in the initial stage in low fertility soils having poor organic matter. Soybean requires relatively large amounts of phosphorus than other crops. The soil should be tested for the availability status of phosphorus to meet the requirement of the crop. With the application of phosphorus the number and density of nodules are stimulated and the bacteria become more mobile. Soybean also requires a relatively large amount of potassium than other crops. The rate of potassium uptake climbs to a peak during the period of rapid vegetative growth then slows down about the time the bean begins to form. Soil test is the best guide for the application of potash in the soil. In the absence of soil test, 50-60 kg $K_2O$ per hectare should be applied. The fertilizers should preferably be placed, at sowing time, about 5-7 cm away from the seed at a depth of 5-7 cm from seed level.

**Water Management**

The soybean crop generally does not require any irrigation during Kharif season. However, if there were a long spell of drought at the time of pod filling, one irrigation would be desirable. During excessive rains proper drainage is also equally important. Spring crop requires about five to six irrigation.

**Harvesting**

The maturity period ranges from 50 to 140 days depending on the varieties. When the plants reach maturity, the leaves turn yellow and drop and soybean pods dry out quickly. There is a rapid loss of moisture from the seed. At harvest, the moisture content of the seeds should be 15 per cent. Harvesting can be done by hand, breaking the stalks on the ground level or with sickle. Threshing can be done either with the mechanical soybean thresher or some conventional methods.

**Nucleus seed production**

***Base population***

- In soybean, a minimum of 500 plants should be selected for planting progeny rows.
- The actual number of plants to be selected will depend upon seed multiplication ratio and targeted quantity of breeder seed.
- The plants selected uniformly from the entire nucleus or breeder seed plot or AVT II seed grown in one acre (0.4 ha).
- The source of base population for pre-released varieties may be plots of Advanced Varietal Trail II.

***Selection from base population***

The selection of plants is done on the basis of morphological identity to the original characteristics of the variety. The selected plants should be true-to-type of the variety.

***Harvesting and threshing of single plants***

- The plants are harvested at the time of maturity and dried for two to three days in the field. Thereafter harvested plants are tied in bundles of 50-70 plants and allowed to shade dry for one week.
- Individual plants are threshed manually to avoid the mechanical damage.
- The seed is kept in paper bags and dried up to 9 per cent moisture before storage.

***Table examination of seed***

- Individual plant seeds are examined for seed characteristics *viz*. seed colour, seed size, seed shape, seed lusture and hilum colour.
- The seeds of any plant not confirming to the standards are rejected.
- Hilum colour in soybean is subject to the occasional variation, therefore any variation from original variety should be especially looked for and discarded.
- The seed with poor appearance and showing symptoms of diseases should also be rejected. Properly labeled seed packets are kept in cloth/ gunny/ polythene bags and stored at 25 $^0$C and 50-60 % RH.

**Breeder seed production**

***Seed source***

It could be nucleus seed stage I or nucleus seed stage II. In exceptional cases, breeder seed stage I can be used to produce breeder seed stage II provided the genetic purity is maintained.

***Isolation distance***

Soybean is highly self-pollinated crop with out-crossing of less than 1%. The minimum isolation distance required is 3m for avoiding the physical mixture.

***Seed rate***

For seed purpose a population of 3.2 to 3.6 lakhs/ha is appropriate. Hence only 80% of the commercial seed rate is to be used.

***Roguing***

- The breeder seed plot should be monitored minutely throughout the crop season specifically at flowering stage, pod filling and maturity stages.
- The off type plants are identified on the basis of characters mentioned in table 1 under supervision of the plant breeder and rogued out.

- All standard packages of practices must be followed with regular observation of crop throughout the growing season.

***Grow out test***

The breeder seed must confirm to the strict standards of genetic purity and subjected to grow out test as per the standard procedure. The plants should be observed for various characters throughout the growing season. The off type plants are tagged and their number should be recorded.

***Packaging, labeling and storage***

- The breeder seed should be dried to 8-9% moisture content and packed in moisture proof bags of 30-40 kg capacity. Bags should be properly labeled and stitched.
- A cool and dry store is recommended having temperature between 20-25 $^{o}$C and relative humidity 50-60%.
- At this storage condition the seeds can be stored safely for 8-9 months.

**Foundation and certified seed production**

***Land requirement***

The field should be well drained and soybean was not grown in the previous season unless the previous crop was from certified seed of the same variety.

***Isolation requirements and other cultural practices***

The minimum isolation distance required is 3m for avoiding the physical mixture.

All standard packages of practices must be followed with regular observation of crop throughout the growing season.

***Rouging***

- Start roguing of the plants affected by yellow mosaic virus and soybean mosaic virus as soon as they appear in the field, so as to check further spread up to first two and three weeks. Continue the removal of mosaic affected plants up to last.
- At flowering stage remove off type plants on the basis of plant characteristics and flower colour.
- Do final roguing at maturity stage, to rogue out off type plants on the basis of pod characteristics.

***Precautions during harvesting and threshing of soybean***

- Harvest the crop when leaves and pods are completely dried.
- Harvested crop should be stacked in small heaps preferably on a cemented pukka threshing floor.

- Threshing can be done when pods become brittle and break upon pressing.
- Breakage of seed coat must be avoided during threshing, generally stationary thresher at a low cylinder speed and stationary combines are used for threshing.
- Small scale produce can be threshed by beating the harvested plants on the plank, as is usually done in paddy threshing.
- Threshed produce should be dried properly in the sun by spreading it in a thin layer.
- The dried produce should be stored in gunny bags and kept on wooden racks in a ventilated, dry store.
- Do not let rain wet the produce.
- Do not store the threshed produce without proper drying

***Seed yield***

Average yield from a good crop of soybean varies between 22-25 quintals per hectare.

Seed production in soybean has **several problems**. Soybean seed is classified in the least storable group. Besides its inherent low viability, soybean seed is also highly prone to the mechanical injury during processing and transportation. It is difficult to maintain the minimum germination standards (70%) till next season. The seed multiplication ratio of soybean is (1:10) i.e. very low leads to high seed requirement, forms the major bottleneck in augmenting the availability of the quality seed.

***Seed standards****

| **Factor** | **Standards of each class** | |
|---|---|---|
| | **Foundation seed** | **Certified seed** |
| Pure seed (minimum) (%) | 97.0 | 97.0 |
| Inert matter (maximum) (%) | 3.0 | 3.0 |
| Other crop seeds (maximum) (per kg) | None | 0.05 |
| Weed seeds (maximum) (per kg) | 5 | 0.10 |
| Germination (minimum) (%) | 70 | 70 |
| Moisture (maximum) (%) | 12.0 | 12.0 |
| Field Standards | FS | CS |
| Off- types | 0.10 | 0.20 |
| Other crop plants | -- | -- |
| Objectionable weed plants | -- | -- |

*As per Indian minimum seed certification standards

# PRINCIPLES AND PRACTICES OF SEED PRODUCTION IN SUGARCANE

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The purpose of applied plant breeding is to develop better varieties. But the benefits of improved new varieties will not reach the farmer unless good quality seed of the variety is produced and supplied in sufficient quantities in a shorter time. Good quality seed can give 10-15% higher yield in most crops. Continued production of good quality seed of the new variety is further required for sustaining the high production potential of the variety. It is to be ensured that the seed produced is genetically pure and free from diseases and pests to realize the full potential of the variety over a longer period of time. Sugarcane, being a vegetatively propagated crop, tends to accumulate disease and pests during cultivation. Production of quality seed free from seed borne diseases and pests is essential to sustain productivity of the crop. Traditionally the breeder seed in sugarcane is produced through heat treatment of the planting material which can eliminate grassy shoot and ratoon stunting disease and partially control smut. However heat treatment cannot control viral diseases like Yellow Leaf Disease for which tissue culture is resorted to. The seed multiplication ratio in sugarcane is generally low 1:10, although it could be-increased through spaced transplanting (STP) method and micropropagation. Foundation seed and certified seed can be produced from the meristem derived plants, provided the cost of tissue culture seedlings can be brought down. If the three-tier nursery programme is properly implemented with initial seed source of tissue culture derived planting materials, it would be possible to rejuvenate old varieties, improve the productivity of present varieties at shorter intervals and improve the sugar recovery. A brief note on seed cane standard, three-tier seed production and profitability of seed cane production is presented in this lecture notes.

## Maintenance Breeding

The ultimate goal of crop improvement programme is the development and release of a higher and stable performing cultivar. According to the International Code of Nomenclature of Cultivated Plants (1969) a variety or cultivar is defined as "*an assemblage of cultivated plants which is clearly distinguished by any character (morphological, physiological, cytological, chemical or others) and which, when reproduced (sexually or asexually), retains its distinguishing characters*". Evolving sugarcane varieties involve a consistent and strenuous effort generally spanning over 12 – 14 years. The first six to seven years go in selection of superior clones at respective research stations

and the later half is spent on evaluating the clones in comparison to the existing or ruling varieties under All India Coordinated Research Project at different locations in any particular zone. After such an enormous efforts a sugarcane breeder is in a position to identify a promising improved clone that is fit to be released as a variety for wide spread.

The general seed production system being followed in India involves three classes of seed, i.e. breeder seed, foundation seed and certified seed. Certified seed is sold to the farmers to raise the commercial crop. Continuous availability of high quality nucleus seed is required to sustain this seed production programme. *Maintaining a high quality nucleus seed is broadly termed as Maintenance Breeding.* Therefore, systematic production of nucleus seed and care during subsequent seed multiplication stages will ensure availability of good quality seed to the farmers to reap better harvest.

Proper maintenance of a variety is important to ensure that it retains its yield potential and other desirable attributes. Maintenance Breeding serves two purposes:

1. It helps in purification and maintenance of a variety and consequent production of nucleus seed.
2. The use of nucleus seed in turn reduces the amount of rogueing required in large breeder seed production plots. In-fact some off-types can be detected only at nucleus seed production stage.

**Maintenance breeding programme for sugarcane varieties**

The benefit of new varieties will not be realized with initial distribution of improved types to farmers and unless provision is made to maintain varietal purity, much of the efforts spent in developing improved types may be lost. Sugarcane being a vegetatively propagated crop warrants special attention for seed because most of the important diseases like red rot, smut, wilt GSD and RSD are transmitted mainly through seed. The following steps are proposed for Maintenance Breeding in sugarcane:

1. Select 2500 (approximately for one acre) true to type canes from about 8 – 10 month old crop plot of the concerned variety. Good cane should be selected, preferable from different clumps, depending upon the quantity of seed to be produced and hence the area to be planted. Materials should be selected from healthy area/rows.
2. Harvest the canes separately. Examine the individual canes for uniformity (true to type to the variety). Reject the canes that are not representative of the variety.

3. Grow cane-to-row progenies (single budded setts may be used). The area under nucleus seed would depend on the requirement of nucleus seed for that particular variety.
4. Examine individual cane rows for genetic purity periodically throughout growing season.
5. Reject entire cane row showing mixture or non-uniformity even on the basis of a single off-type clump in the progeny. Such rows should be rejected and uprooted as and when detected.
6. The diagnostic characters and their range of variation help in identification of off-types. Diseased as well as agronomically poor looking rows should also be rejected.
7. Progeny rows true to type (phenotypically) of the original variety for all externally observable characters are further selected harvested separately and examined.
8. Bulk the harvested true to type seed, which is called as Nucleus Seed.
   *Purity standards:* The nucleus seed so produced should conform to the highest standard of purity, approaching 100 per cent. There should not be any compromise on this point.

**Seed Cane Standards**

The standard for seed cane quality was first published by the Indian Standard Institution (ISI) on December, 1966 (ISI No: 3866-1966: *Specification for Sugarcane Seed Materials*). Later, to formulate standards for seed cane to fit into the Seed Act, a task force was constituted in 1978 under the chairmanship of Dr. Kishan Singh, former Director of IISR, Lucknow. After several round of discussion, the revised seed cane standard was finalized and a draft was published in 1990 by the IISR as "*Standards for Sugarcane Seed Material*" (Technical Bulletin No 25). Again, in 1999, the field and seed standards for sugarcane planting material was reviewed by a committee constituted by the ICAR, New Delhi. The revised draft was approved by the Technical Committee of Central Seed Certification Board in October 2001 and later notified by the Central Seed Certification Board. The approved seed cane standards along with general seed certification standards as applicable for sugarcane are presented below.

***A) General seed cane certification standards***

***1. Classes of seeds***: As followed in other crops, four generation systems of seed multiplication namely, nucleus seed, breeder seed, foundation seed and certified seed can be adopted for the conventional system of seed production in sugarcane as shown in Table 2.

**Table 2.** Classes of seeds applicable for sugarcane seed production programme

| **Category of seed** | **Nucleus seed** | **Breeder seed** | **Foundation seed** | **Certified seed** | **Commercial Production** |
|---|---|---|---|---|---|
| **Seed Source** | Research station or breeder who developed it. Use heat treated seed. | Use heat treated seed, if not done at nucleus seed stage. The tissue culture derived planting material is designated as breeder seed. | Produced from breeder seed or tissue culture derived planting material | Produced from foundation seed or tissue culture derived planting material | Produced from certified seed |
| **Place of production** | Research Centre | Research Centre | Govt. Seed Farm, Sugar Factory Farm and progressive farmers' field | Certified seed growers and progressive farmers | Farmers' field |
| **Responsibility for maintaining purity** | Concerned breeder or research Centre | Concerned breeder or research Centre | Research Centre / State Dept. Of Agriculture / State Cane Development Dept. / Sugar factories | State Dept. Of Agriculture / State Cane Development Dept. / Sugar factories | Farmers |
| **Certification** | Exempted | Exempted | Concerned breeder / Expert team / competent | State Seed Certification Agency | Not always |

| | | | | | |
|---|---|---|---|---|---|
| | | | authority | | |
| **Remarks** | The explants for tissue culture are taken from nucleus seed. | Primary nursery is raised from breeder seed | | | Replace the certified seeds after 3-4 cycles of crop with fresh lot of certified seeds |

**2. Certification procedures and Seed cane standards**

***A. Sampling and field counts****:* Five counts may be taken up to an area of 5 acres and an additional count is taken for each additional 5 acres (Chowdhury, 2007). Number of plants observed in one count is 100.

***B. Appearance and physical purity****:* Physical purity of seed should be 98 per cent. Seed cane should be undamaged and reasonably clean. The crop should not have more than 10 per cent lodged canes. De-trashing is not recommended for seed crop. The maximum permissible limits for the striping of dry foliage shall be 2.0 %. Seed canes should not have aerial roots / nodal roots. In water-logged areas relaxation may be given up to a maximum of 5 per cent.

***C. Sett moisture content****:* Moisture in seed cane should not be less than 65 per cent on wet weight basis.

***D. Genetic Purity****:* The seed material shall be of only one variety. No admixture is permitted. The genetic purity of seed cane should be 100 per cent.

***E. Germination****:* Germinability of buds should not be less than 85 per cent.

***F. Bud quality:*** Each node of a seed cane shall bear one viable bud. The number of nodes without sound buds shall not exceed 5 per cent (by number) of the total number of buds in a stalk. The number of buds which are swollen or have projected beyond 1 cm from the rind surface shall not exceed 5 per cent (by number) of total number of buds.

***G. Seed Source****:* The certified classes will be produced from seed cane and/or mericlones whose sources and identity may be assured and approved by the certification agency.

***H. Land Requirements****:* A seed crop of sugarcane shall not be eligible for certification if planted on land on which sugarcane was grown in the previous

season. Land/seed crop shall be kept free from sugarcane residues and drainage from other sugarcane fields.

***I. Heat Treatments***: Nucleus or Breeder stage shall be raised from heat-treated seed cane.

***J. Field Inspection:*** A minimum of three inspections shall be made as under:

- **Stage-I**: The first inspection shall be made at 45-60 days after planting to verify isolation and detect volunteer plants, designated diseases and pests and other relevant factors.
- **Stage-II**: The second inspection shall be made at 120-130 days after planting to verify off-types, designated diseases and pests and other relevant factors.
- **Stage-III**: The third inspection shall be made 15 days prior to the harvesting of seed canes to verify the age of cane, off-types, designated diseases and pests and other relevant factors.
- Whenever, the off-types and diseased plants are noticed it should be rouged out along with roots and destroyed.

***K. Field Standards***

i) ***General requirements-Isolation***: The sugarcane seed production fields shall be isolated from other fields with a minimum distance of 5 m to avoid mechanical mixture of other varieties.

*ii)* ***Specific requirement***

| **Particulars** | **Stage of field inspection** | **Maximum permissible limit %** | |
|---|---|---|---|
| | | Foundation seed | Certified seed |
| **Off-type** | I, II, III | None | None |
| **Plants affected by designated diseases** | | | |
| *a) Red* rot: *Glomerella tucumanensis* Speg. Arx & Muller / (*Colletotrichum falcatum*) | I, II, III | None | None |
| b) Smut: *Ustilago scitaminea Sydow.* | I<br>II<br>III | 0.02*<br>0.01*<br>None | 0.10*<br>0.10*<br>None |
| c) Grassy shoot: Caused by MLO | II<br>III | 0.05*<br>None | 0.05*<br>None |
| d) Wilt: *Cephalosporium sacchari* Butler. | III | 0.01* | 0.01* |
| Leaf scald: *Xanthomonas albilineans.* (Ashby) Dowson | II<br>III | 0.01*<br>None | 0.05*<br>None |
| **Plants affected by designated insect pests** | | | |
| a) Top borer : *Scirpophaga excerptalis* | II & III | 5.0 | 5.0 |

| | | | |
|---|---|---|---|
| walker | | | |
| b) Internode borer : *Chilo sacchariphagus indicus* Kapur | III | 10.0#<br>None** | 20.0<br>None** |
| c) Stalk borer: *Chilo auricilius* Dudgeon | III | 20.0+<br>None** | 20.0<br>None** |
| d) Plassey borer: *Chilo tumidicostalis* Hampson<br>Gurudaspur borer : *Acigona steniellus* Hampson<br>Scale insect: *Melanaspis glomerata* Green<br>Mealy bug : *Sacchariphagus sacchari* Cockerell | III | 5.0<br>None** | 5.0<br>None** |

# Around 10% affected internodes. * Subject to immediate rouging of the whole clump

+ Around 0.5% affected internodes. ** In area where the presence of the pest has not been recorded.

A well set seed production and certification programme is available for crops like rice, wheat, pulses, oilseeds, etc but it is a grey area in sugarcane or not followed in strict sense because of the bulkiness of seed cane, transportation and packing problem and short shelf life of setts. The quantity and cost of sugarcane seed used for planting a unit area is high as compared to other crop (Table 3). Ram (2007) pointed out that the bulkiness and high cost of sugarcane act as a bottleneck in providing quality seeds to farmers. The age of the seed crop prescribed for sugarcane is 8-10 months for subtropics. To have such crop, the seed crop has to be planted during May-July. But planting in May result in poor yield in North India and July planting is not practiced due to rainy season. Another issue is packaging and labeling. The Certification agency would be reluctant to certify the crop because during transport of seed cane quality loss may happen.

**Table 3.** Seed rate and seed cost of sugarcane and other crops

| **Crop** | **Seed Rate (kg/ha)** | **Approx cost of seeds per kilo** | **Cost of seed/ha** |
|---|---|---|---|
| Wheat | 100 | 25.00 | 2500 |
| Paddy | 40 | 30.00 | 1200 |
| Gram | 30 | 50.00 | 1500 |
| Sunflower | 10 | 50.00 | 500 |
| Sugarcane | 10000 | 2.30 | 23000 |

The maximum permissible limit for stripping of dry foliage shall be 2% but some of the self-detrashing varieties may have higher % of stripped leaves (Ram, 2007). Strong winds during post monsoon season are common in sub-tropical India. This would cause lodging and it may exceed the permissible limit

of 10%. Inspection/monitoring of seed cane at certified seed stage is difficult hence in strict sense, certification is not done in all seed production plot. Alternatively, the staff of the seed certification agency, sugar mills and agriculture department can be trained for the job so that at least foundation seed and randomly selected certified seed plots are inspected and certified. This sort of approach is required for the maintenance of varietal purity and management of seed borne disease.

**Thermotherapy**

Vegetative propagation of sugarcane through setts favours accumulation of endophytic and ectophytic microbes like phytoplasma, viruses, fungi and bacteria in seed canes. Some of these microbes are pathogenic while others may cause stunted growth or clonal degeneracy under non-normal situations. Slow accumulation of harmful microorganism in seed canes over a period of time makes minor disease into major one. Movement of seed cane from infected area may inadvertently carry a new pathogen or new strain of an existing pathogen or both hence, along with seed canes disease causing microbes are also introduced into a new area. For a sustained sugarcane production in the country, disease free seed production programme is a must. Thermotherapy is a practical approach to eliminate the pathogens resides in seed cane. Thermotherapy is a process of killing / inactivating the pathogen present in sugarcane setts by subjecting the setts to different kind of heat treatment at specified temperature and for a specified duration without affecting the germinability of buds. Four types of thermotherapy are recognized namely, 1) Hot water treatment, 2) Hot air treatment (HAT), 3) Moist hot air treatment and 4) Aerated steam treatment. The details of MHAT and AST, most commonly used, are described below.

***1 Moist hot air treatment***: The moist hot air (MHAT) treatment is followed in India particularly, in the subtropical region. The MHAT plant is more or less similar to HAT plant, except that instead of dry hot air, moist hot air is circulated inside the treating chamber under high humidity condition. The MHAT plant consists of a boiler unit, temperature control panel, air circulating fans and a chamber for placing full length canes. The capacity of the plant may vary from 5-15 quintals. Full length seed canes (immature or mature canes) after removing tops and trashes are spread evenly in single layer over the perforated iron trays. These trays are arranged in their respective slot one above another in the treating chamber. After closing the doors of the treating chamber moist hot air is circulated inside. The canes are treated at 54 $^{o}$C for 2 hrs. Moist hot air treatment is effective against GSD and RSD. Although there is no adverse effect of moist hot air treatment on germination, it is recommended to

treat the treated setts with fungicide before planting to prevent the entry of soil pathogen into the setts.

***2 Aerated steam treatment (AST)****:* AST is widely used in sugarcane growing countries. The AST plant consists of a steam generating chamber, air-blower, temperature controller panel, mixture chamber and treating chamber. The treatment chamber can hold 18 perforated GI sheet trays of size 60x30x30 cm. Each tray can accommodate 150 setts. Steam is generated by heating water. This steam is passed to another chamber where it is mixed with air in the proportion of 1:4. This aerated steam is passed into the cane treating chamber. Setts are treated at 50 $^{o}$C for about 2 hr. AST is effective against GSD, RSD, and partially against smut but not effective against red rot.

**Micropropagation**

Sugarcane being a vegetatively propagated crop, deterioration of yield and quality is inevitable prior to commercial use on account of systemic infections of viruses and bacteria during vegetative multiplication. Viruses that are of concern in the global sugarcane growing areas are Sugarcane mosaic virus (SCMV) and Sugarcane yellow leaf virus (ScYLV) which spread systemically in plants and are seed borne. It was found that severe infection of the viruses causes 30 - 50 % in yield reduction. The sustained high yield and production of sugar per unit area depends primarily on good quality seed cane, which has to be genetically pure and free from diseases. As there are no control measures by which virus infection can be eliminated from an infected plant, the only means of avoiding losses due to viral disease is to start with tissue culture plantlets derived from apical meristem tip culture which are virus free. Plant tissue culture is the best methodology through micropropagation for production of quality and disease free planting material at a faster rate in a shorter period of time. The production of quality seed through micropropagation technique is well recognized now.

***Tissue culture based quality seed production programme***

At ICAR-Sugarcane Breeding Institute the nucleus clones of released varieties viz., Co 86032, Co 0212, Co 06030, Co 06022 and Co 0403 were maintained in seed chain under the supervision of the breeders. The varieties were micro-propagated to supply disease free plantlets for further multiplication as Breeder seed at the institute. Anticipating the release of the newly proposed variety Co 09004 to CVRC, this clone also included. Through apical meristem tip culture, the varieties Co 86032, Co 0212, Co 0238, CoM 0265, Co 06022, Co 06030 and Co 11015 was multiplied *in vitro* and tissue culture plants were supplied to the sugar factories and progressive farmers.

***Supply of mother culture flask to tissue culture laboratories***

Sugarcane micropropagation involves three stages which include culture establishment, shoot multiplication and root development. The first stage i.e., shoot regeneration from the meristem tip is time consuming. From multiple shoot formation stage (mother culture), further multiplication and rooting will be easy and faster for the private tissue culture laboratories. Virus indexed mother culture flasks of varieties Co 86032, Co 0212, Co 0238 and Co 11015 were supplied to tissue culture laboratories of Tamil Nadu, Karnataka, Gujarat and Maharashtra.

Micropropagated plants have been well accepted by sugar factories and farmers all over the country because of its uniform productivity, free from disease, vigourous growth and high yield. The technology, is successfully incorporated into the system of seed multiplication in sugarcane wherein the basic clean tissue culture plantlets forms the starting planting materials from which the single budded settling or two budded setts are used for raising primary seed nursery and secondary seed nursery after treating with fungicide, insecticide and nutrients using new sett treatment device developed by ICAR-SBI and ICAR-CIAE which enhances germination and quality settlings. Seed replacement once in five years with disease free quality planting material is important. Adopting tissue culture based seed nursery programme has impact on higher cane yield and sugar recovery. It is possible to achieve 20-25 tonnes/acre additional yield through the improved tissue culture based quality seed producton. Thus, enhancing sugarcane productivity and making the sugar industry more profitable in the future.

**Three Tier Seed Nursery Programme**

Since, sugarcane is propagated through vegetative cuttings, clonal degeneracy is quite common after repeated cycle of propagation or years of cultivation. Over the years, vigour of the clone is reduced due to accumulation of microbial load. Disease like ratoon stunting (RSD), grassy shoot (GSD) and smut are seed borne diseases and known to be carried forward through seed cane. Therefore, elimination of these diseases and raising healthy nursery crop should be an important objective of cane development / seed production programme. A properly designed seed production system is must *i.e.* a system through which seed borne diseases are eliminated or its spread is minimized and at the same time quality, vigour and production potential of a variety could be maintained over a longer period. This is done through a practice known as three-tier nursery programme which consists of primary seed nursery, secondary seed nursery and commercial nursery. This programme is mainly based on the heat therapy because of its effectiveness in controlling seed borne infections. The 3-tier nursery programme also encompasses various prophylactic measures to control insect pests, thus ensuring healthy seed cane. In this system, heat

treated setts are planted in primary nursery followed by further multiplication in secondary and commercial nursery. The seed canes harvested from commercial nursery are supplied to farmers for commercial planting. This system of seed multiplication is similar to the three-generation seed multiplication system, except that primary nursery is raised from the breeder seed and it is strongly recommended for each sugar mill to follow this system in their mill zone area. Further details of the 3-tier seed nursery are given below.

***1. Primary nursery****:* The primary nursery is raised from breeder seed obtained from the research station. The crop is raised in the sugar mill farm / State seed farm itself after heat treatment in any of the methods mentioned under Thermotherapy. In South India aerated steam therapy is used whereas in North India, moist hot air therapy is adopted. After heat treatment, the setts are dipped in solution containing fungicides (Carbendazim 0.1% or Bayleton 0.1%) and insecticides (Imidacloprid or Chlorpyriphos @ 1ml/lit of water) to protect the cut ends from invasion of soil-borne diseases like pineapple disease, wilt, etc. The crop should be given good nursery management practices including higher dose of FYM and fertilizers. Adopt 25% higher seed rate at primary nursery to compensate germination losses due to heat treatment. Rogue out the diseased, infested plants and off-types. Harvest the crop at 6-10 months and distribute the seed canes to selected farmers for raising secondary nurseries. From primary to secondary nursery the seed multiplication ratio would be around 1:7.

***2. Secondary nursery****:* Seed from primary nursery are distributed to progressive farmers to raise secondary nursery. The crop is maintained and monitored as it is done for the primary nursery. Harvesting of seed is done at 8-10 months and distributed to farmers for raising commercial seed nurseries. From secondary to commercial nursery the expected seed multiplication ratio is 1:8.

***3 Commercial nursery***: Commercial nurseries are raised from secondary nursery and maintained and monitored like secondary nursery. Seed can be harvested after 6 months and continue till 12 months. The multiplication rate is about 6-8 times.

***4 Seed nursery programme for sugar mills***: Once setts are heat treated at primary nursery, the setts will be free from grassy shoot disease (GSD) and ratoon stunting disease (RSD) for about 5 years (Chart 1). Therefore, a well planned seed nursery programme such as dividing the mill zone area into 5 sectors and then replacing old seed in each sector in every year or the whole zone in a span of 5 years is a must to maintain constant flow of good quality seeds materials among farmers in each mill zone. Alexander (1995) has

presented a heat treated nursery programme to cover 10,000 ha area of the factory. This is reproduced below. The factory area of 10,000 hectare is divided into 5 sectors of 2,000 ha each. In the first year, 20 ha in sector I is planted with AST treated setts. In the second year, the cane in the 20 ha area in Sector I is multiplied to 200 ha (10 times), and simultaneously 20 ha in sector II is planted with AST treated setts. In the third year, cane in the 200 ha area in sector I is multiplied to 2000 ha, thus covering the entire sector. Cane in the 20 ha area in sector II is multiplied to 200 ha. Simultaneously 20 ha in sector III is planted with AST treated setts. Thus the cycle is repeated covering the entire factory zone of 10,000 ha once in five years. At each stage careful inspection and rogueing is necessary. Under proper supervision, the treated material needs to be changed once in five years.

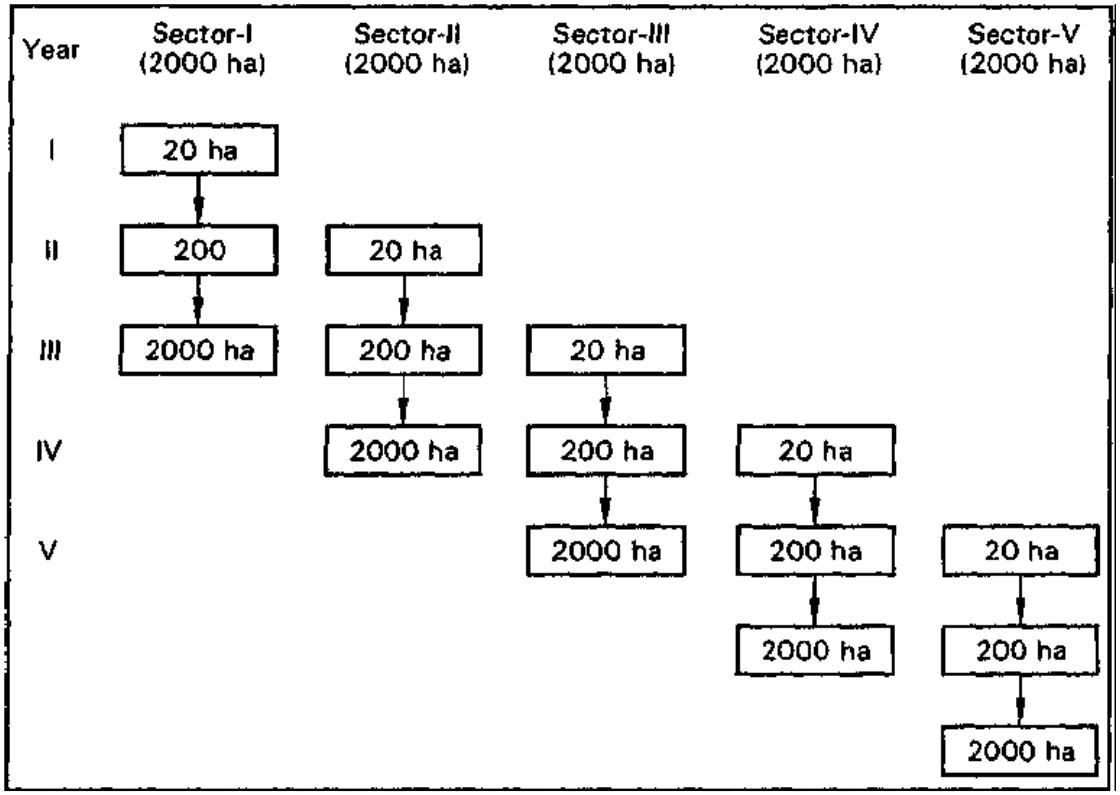


**Chart 1.** Planting schemes to cover 10,000 ha in 5 years using the heat treated seeds

Naidu (2010) has presented an *integrated seed nursery programme* for producing disease free planting materials in short time and at reasonable cost (Chart 2). This approach involves integrating advance technologies such as tissue culture technique, virus indexing method with budchip technique. The integrated seed nursery programme involves four tier nursery programme namely, breeder seed nursery (raised with tissue culture seedlings), primary nursery, secondary nursery and commercial nursery. Naidu (2010) has proposed two version of *integrated seed nursery programme*, the first one uses

tissue culture seedlings, budchip settlings and two budded setts. The multiplication cycle start from breeder seeds and pass through primary, secondary and commercial nursery. The second version of *integrated seed nursery programme* uses only tissue culture seedlings and budchip settlings. In this method, the requirement of secondary nursery and commercial nursery are dispensed with.

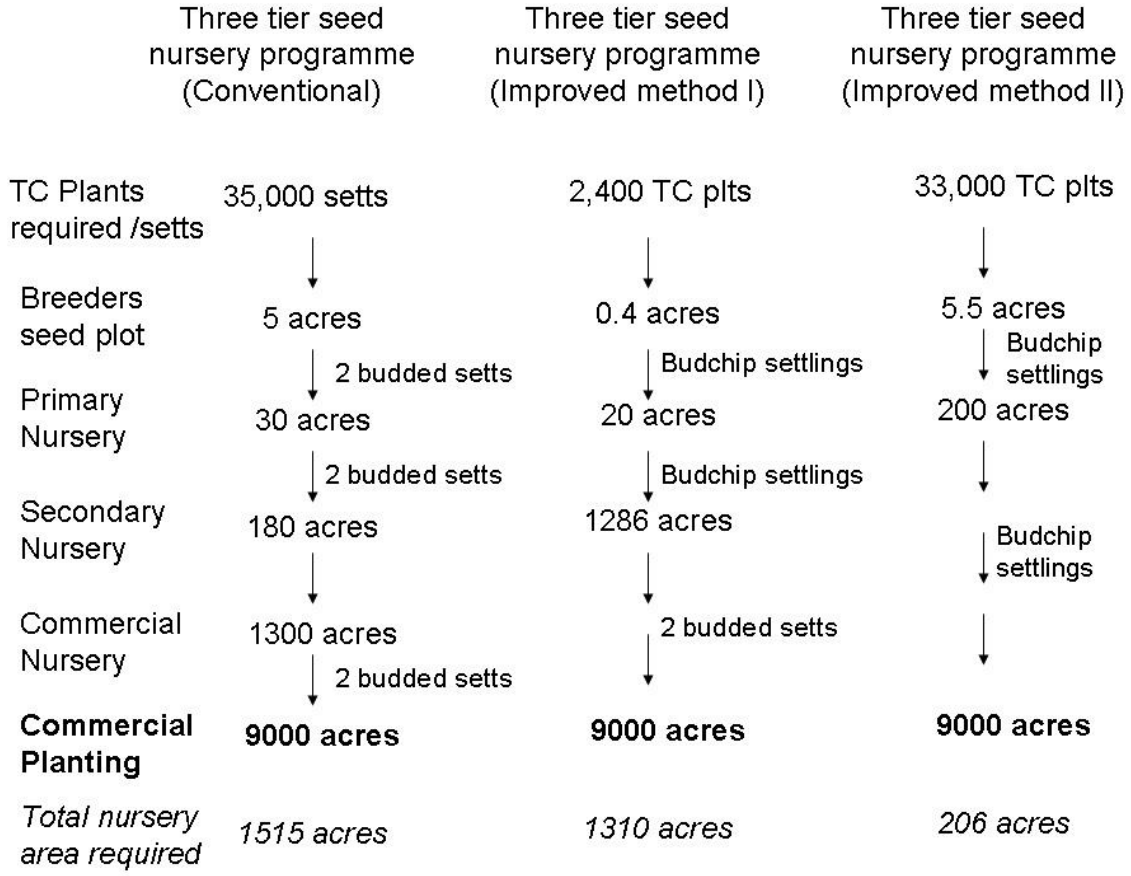


**Chart 2.** An integrated seed nursery programme for a sugar factory of 2500 TCD

**Problems in Sugarcane Seed Production Programme**

***1. High cost and quantity of sugarcane seed:*** The cost and quantity of seed used for planting one hectare of area is very high in comparison to other major crops. Both these items are acting against the successful seed programme in sugarcane.

***2. Age of seed crop*:** As per the Seed Cane Standards for sugarcane the age of seed crop shall be 8 – 10 months at harvest in the sub-tropics. To have such a seed, the crop has to be planted during the month of May. May planting of seed crop would result in poor yields when harvested during next March. It will further add to the already high cost of sugarcane seed. Further, it will not be

feasible to have 8 – 10 month sugarcane crop during May for planting seed crop to complete the seed chain in sugarcane.

The crop has to be planted during July August to get seed of 10 month old crop for planting seed crop during May (main season), which is not possible as July and August are rainy months in sub-tropics. Further, yields of such July – August planted crop will be very low when harvested in May.

***3. Problems in seed certification:*** Considering the uniqueness of sugarcane crop it will not be possible to meet certain standards / requirements like packing and labeling, grow out test etc. of seed certification. Without packing and labeling of sugarcane seed nobody would like to take the responsibility of events happening during transportation of seed from one place to another.

The maximum permissible limits for the stripping of dry foliage shall be 2.0% may not hold good in certain self-stripping sugarcane varieties. Further, strong winds during post-monsoon season are common in sub-tropics, which cause extensive lodging in sugarcane and will surpass the permissible limit of 10% lodged canes.

***4. Non-availability of nucleus seed at the time of identification and release of variety:*** Normally the breeder starts nucleus seed production of a variety only after the variety gets identified for release with the result that there is no nucleus seed available to take up breeder seed production in the very first year of release of a variety. It is suggested that once the material reaches final year of advance varietal trial the concerned breeder should initiate the nucleus seed production. This would be the starting point of Maintenance Breeding and should be continued once a variety has been identified and released for general cultivation.

**Research Need for Seed Production in Sugarcane**

1. There is need of research on Seed Technology aspects in sugarcane. As such, guidelines on sample size for estimating various seed quality / purity parameters (for example number of swollen buds, stripping of dry foliage, nodal roots moisture content, germinability of buds etc) are lacking in sugarcane.
2. As per the seed cane standards the age of the seed cane crop at harvest for seed purpose shall be 6 to 8 months and 8 to 10 months for planting in tropics and sub-tropics, respectively. It is not feasible to maintain the seed crop age of 8 to 10 months in the seed chain in sub-tropics. Hence, there is need to study the germinability of seed at an age of 6, 8, 10 and 12 months. If there is insignificant difference in germination % between 10 and 12

months then the age of seed cane shall be 12 months in sub-tropics. The feasibility of compensating the lower germination of 12 month old crop by increased seed rate shall also be explored.

3. Generally the seed multiplication ratio in sugarcane is 1:10, which is low in comparison with other major crops. There is need to explore and adopt new technologies to increase the yield of seed crop. This is more important in view of the age (8 to 10 month) of the seed crop, especially in sub-tropics.
4. Technologies to reduce the seed rate need to be developed and adopted in order to reduce the cost of seed.
5. There is need to change in seed certification standards in the absence of packing and labeling of sugarcane seed in order to bring the sugarcane crop under the preview of seed certification.

**Cultural Practices for Sugarcane Seed Production**

**Seed treatment:** The selected canes are subjected to heat treatment in order to kill the seed borne inoculum. The heat-treated canes should be sized (individually) in one or two budded setts. Further to increase the efficiency of disease control the setts to be treated with 0.1 % Bavistin or a mercurial compound. The setts have to be dipped in 0.1% Melathion solution also. Now the setts of individual canes are ready for planting.

***Land requirement:*** A seed crop of sugarcane shall not be eligible for certification if planted on land on which sugarcane was grown in the previous season. Land / seed crop shall be kept free from sugarcane residues and drainage from other sugarcane fields.

***Isolation:*** The sugarcane seed production fields shall be isolated from other fields with a minimum distance of 5 m to avoid mechanical mixture of other varieties.

***Planting of seed:*** The planting of setts of individual canes should be done in rows. The row length suggested is 1.5 - 2.5 meters depending upon the length of canes with a spacing of 90 cm between rows. After every block a path of 0.50 – 0.75m is required. This system of planting will help better and easy inspection and monitoring of the crop from time to time by concerned scientists.

***Inspection of seed crop*:** Examine the seed crop for genetic purity periodically throughout growing season. A minimum of three inspections at 45 – 60 days and 120-130 days after planting, and 15 days prior to the harvesting of seed canes shall be made for the purpose as explained earlier.

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# PRINCIPLES AND PRACTICES OF SEED PRODUCTION IN OILSEED BRASSICAS

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Indian subcontinent is the natural repository of the oilseed crops. Brassica species, commonly called as rapeseed-mustard, are the third most important oilseed crops of the world after soybean and palm. China, India, Canada, Japan and Germany are the major rapeseed-mustard growing countries. These are the second most important oilseed crops of India, next to soybean. India is one of the largest rapeseed-mustard growing country occupying first position with 20.23% area and second position with 11.7% share to the global production (USDA, 2012). Four oleiferous Brassica species viz. *Brassica juncea, B. napus, B. rapa* and *B. carinata* are cultivated in about 6.39 million hectares area and produced 7.41 million tons in India (Kumar, Kumar & Kandpal, 2012). Among the oilseed Brassicas {Indian mustard (*B. juncea*); Gobhi sarson (*B. napus*); Ethiopian mustard (*B. carinata*); Yellow sarson, Toria, brown sarson *(B. rapa*); both self and cross pollinated forms are available (Table 1). In India, a good number of improved varieties in these crops have been developed and their improved production and protection technologies were refined to obtain higher yields. Due to different maturity durations (Table 1), these species are suitable for different cropping systems. Further, being diverse these are adapted to different agroclimatic conditions. Among these, *B. juncea* (2n=36, AABB genome) contributes more than 80% to the total rapeseed-mustard production in the country and is an important component in the oilseed sector.

**Table 1:** Mode of reproduction, ploidy level and maturity of oilseed Brassicas

| **Name of the crop** | **Common name** | **Ploidy level** | **Compatibility type** | **Days to maturity*** |
|---|---|---|---|---|
| *B. juncea* | Indian Mustard | Amphidiploid | Self-compatible | 100-152 |
| *B. napus* | Gobhi sarson (rapeseed) | Amphidiploid | Self-compatible | 145-180 |
| *B. carinata* | Ethiopian mustard | Amphidiploid | Self-compatible | 160-180 |
| *B. rapa* var. toria | Toria (rapeseed) | Diploid | Self-incompatible | 70-100 |
| *B. rapa* var. Yellow sarson | Yellow sarson (rapeseed) | Diploid | Self-compatible | 90-135 |
| *B. rapa* var. Brown sarson | Brown sarson (rapeseed) | Diploid | Self-incompatible | 110-150 |

*Based on days of maturity among the released varieties

Quality seed is the most important input in realizing higher yields. Once a variety is released it takes about 3-4 seed multiplication cycles to reach the farmers. In addition to the genetic potential, fate of the variety also depends on the quality of the seeds used for commercial cultivation. Therefore, utmost care needs to be taken for maintenance of purity in this process. The process of seed production starts with the production of nucleus seed. The purity of any genotype can be breached at different stages and during different agronomical operations. The factors identified by Agrawal (1980) for variations in the seed crop/harvest are:

- Developmental variations
- Seed admixture during harvesting or threshing or handling
- Mutations
- Natural crossing with undesirable types
- Minor genetic variations
- Selective influence of diseases and insect pests
- Technique of plant breeder

The affect of the above factors can be minimized by strict compliance of seed production procedures and practices defined under section 8 of the Seed Act 1966 ensuring the minimum seed certification standards of any crop variety during seed production. The required level of genetic purity need to be maintained for each class of seeds i.e. nucleus, breeder, foundation and certified seeds. Various field and laboratory standards are to be followed for genetic purity of the variety under seed production chain.

**Field standards**

- Selection of field
- Good agronomic practices
- Isolation distance
- Roguing at appropriate stage

**Seed Standards**

- Pure seed (Min)
- Inert matter (Max)
- Other crop seeds (Max)
- Total weed seeds (Max)
- Objectionable weed seeds
- Germination (Min)
- Moisture (Max)

The procedure of nucleus/breeder/foundation/certified seed production varies from crop to crop according to their mode of pollination. Field and seed standards as per the Indian Minimum Seed Certification Standards (Trivedi and Gunasekaran, 2013) are briefly discussed as under:

**1. Selection of land for seed production**

- Seed crop is taken in a field in which same crop was not taken in the previous season. Failing which volunteer plants are often observed in seed crop
- Shattering of seed at harvest in previous crop and seed dormancy increase the incidence of volunteer plants which are the major source of contamination in seed crop
- Frequency of such plants higher than permissible limit 0.10 and 0.50 for foundation and certified seed respectively makes the seed crop unfit for seed certification
- If however, higher or same class of seed of given variety was taken in the previous year, the seed crop of the same variety can be grown in the field

**2. Field inspections/ Roguing**

**Pre-flowering stage**

- Off-type plants: weeds, plants of different variety/species
- Plant height
- Leaf characters

**Flowering stage**

- Differences in petal colours, plant height, early and late maturing plants

**Pre-harvesting/Maturity stage**

- Late maturing plants
- Size, shape and surface of siliqua
- Angle of siliqua placement
- Pod locules
- Seed colour
- Mexican prickly poppy (*Argemone mexicana*) is an objectionable weed. All plants of this weed should be removed.

Identification of off-type plants in a seed production plot is very crucial which require lot of skills. The off-types may be a weed or a plant of different variety/ species, need to be removed at adequate stage. In rapeseed-mustard different diagnostic traits are documented (Yadava and Singh 2003), which are helpful in identification of off-types (Table 2).

**Table 2:** Stage specific diagnostic traits considered for varietal identification in oilseed Brassicas

| Characteristics | States | Stage of observation |
|---|---|---|
| Leaf shape | Serrated / non serrated | Vegetative |
| Leaf type | Sessile / petiolate | Vegetative |
| Leaf colour | Light green / medium green / dark green / purple green / purple | Vegetative |
| Leaf hairiness | Present /absent | Vegetative |
| Calyx colour | Green / light green | Flowering |
| Corolla colour | Dark yellow / yellow / cream yellow / white | Flowering |
| Petal shape | Narrow / broad | Flowering |
| Plant type | Erect / semi-erect / spreading | Vegetative/reproductive |
| Plant height | Dwarf / medium / tall | Vegetative/reproductive- |
| Main shoot length | Short / medium / long | Vegetative/reproductive |
| Siliqua arrangement | Appressed / semi-appressed /spread | Maturity |
| Siliqua surface | Smooth / intermediate / constricted | Maturity |
| Siliqua beak | Short stout / long slender | Maturity |
| Seeds / siliqua | Less / average / more | Maturity |
| Pod locule | Unilocular / bilocular / trilocular / tetra locular | Maturity |
| Seed colour | Yellow / dull grey / reddish brown / brown / black | Maturity/post harvest |
| Seed size | Small / medium / bold | Post harvest |
| Oil content | Low / medium / high / very high | Post harvest |

## 3. Field Standards

### A. General requirements

**Isolation distance:** For the purpose of seed production, the self pollinated types (compatible types) should be considered as often cross pollinated crops since the natural out crossing from 5 to 15 per cent is reported. The natural out crossing is largely facilitated by honeybees in both self compatible and incompatible types. The isolation distance for both types is as follows:

| Mode of pollination | Foundation seed | Certified seed |
|---|---|---|
| Self-compatible types | 100 m | 50 m |
| Self-incompatible types | 200 m | 50 m |

**Seed field to be isolated from:**

- Field of other varieties of same species
- Field of same variety not conforming to varietal purity requirements
- Field of *Eruca sativa* and other *Brassica* species: *B. rapa, B. chinensis, B.napus, B. juncea, B. nigra, B. pekinensis, B. tournifortii, B. alba*

- No isolation is required from *B. oleracea* types (cabbage, cauliflower, knol khol etc.)

**B. Specific requirements**

| **Factor** | **Maximum permitted (%)*** | |
|---|---|---|
| | **Foundation** | **Certified** |
| Off types | 0.10 | 0.50 |
| Objectionable weed plants | 0.05 | 0.10 (*Argemone maxicana*) |

*Maximum permitted at the final inspection

**4. Seed standards:**

There are no fixed seed standards for nucleus and breeder's seed as these are produced by the breeder himself along with the representatives from ICAR, NSC, therefore, their genetic purity should be of highest level. However, for certification of foundation and certified seed standards has been laid out, which are mandatory for qualification of seed. The seed standards for rapeseed-mustard are as follows:

| **Factor** | **Maximum permitted (%)*** | |
|---|---|---|
| | **Foundation** | **Certified** |
| Pure seed (minimum) | 97.0% | 97.0% |
| Inert matter (maximum) | 3.0% | 3.0% |
| Other crop seeds (maximum) | 10/kg | 20/kg |
| Other distinguishable varieties | 0.10%(by | 0.50% (by number) |
| Total weed seeds (maximum) | 10/kg | 20/kg |
| *Objectionable weed seeds (maximum) | 5/kg | 10/kg |
| Germination (minimum) | 85% | 85% |
| Moisture (maximum) | 8.0% | 8.0% |
| **For vapour - proof containers (maximum)** | | |
| Mustard and taramira | 5.0% | 5.0% |
| Rapeseed | 7.0% | 7.0% |

**Rapeseed-mustard Hybrid seed production:**

Hybrids released for commercial cultivation in rapeseed-mustard are based on cytoplasmic genetic male sterility- fertility restoration (CGMS-FR) system commonly called as 3-line system. The seed of male sterile line (A line) is produced by raising A and B lines in a specific ratio. In general, 4:1 ratio of A line and its maintainer (B line) is followed. To avoid admixture, B line need to be

harvested first and later the A line is harvested and threshed. The seeds of both these lines need to be threshed separately and bulked as separate lots of A and B lines for further use. The hybrid seed through raising A and R (fertility restorer) need to be raised in a ratio of 4:1 to 5:1 depending on the flower bearing capacity of R line. To avoid admixture, R line need to be harvested first and later on A line is harvested and threshed. The seeds from both these lines need to be threshed separately and bulked as separate lots of hybrid ($F_1$) and R line seed. The seeds of B and R lines can be multiplied by growing them separately in isolation just like any other pureline variety.

**1. Selection of land for seed production:** Same as in varieties

**2. Isolation distance for hybrid seed production and multiplication of A/B/R lines**

| **Contaminants** | **Minimum isolation distance (m)** | |
|---|---|---|
| | **Foundation** | **Certified** |
| Fields of the varieties/ hybrids/ parental lines other hybrids of same parental lines of the same spp. | 200 | 100 |
| Fields of the same variety/ hybrid/ parental line not conforming to varietal purity requirements for certification | 200 | 100 |
| Fields of Rocket salad (*Eruca sativa*) and any of the other species of the genus *Brassica*<br>• *B. juncea* (L.) and its sub spps., Indian mustard or rai Bangla sarson or vegetable mustard or Pahadi rai<br>• *B. rapa* cvs. Yellow sarson, toria and brown sarson<br>• *B. napus*: Gobhi sarson; *B. carinata*: Karan rai<br>• *B. nigra*: Black mustard or Banarasi mustard<br>• *B. alba*: White mustard; *B. tournefortii*: Jungli rai | 50 | 25 |

**5. Grow out test:** All seed lots shall be subjected to grow-out test and shall confirm to the following genetic purity requirements:

| **Class** | **Genetic purity (%) (Minimum)** |
|---|---|
| Foundation | 95.0 |
| Certified | 85.0 |

**6. Important agronomic interventions for better seed quality and quantity:**

- The seed production of a variety should be taken up in the area for which it is released
- Sowing should be done on time as per the recommendations for agro-climatic or geographical zone
- Standard row to row and plant to plant spacing should be maintained
- Recommended dose of fertilizers should be applied for raising healthy crop
- Thinning should be done three weeks post-sowing
- Two-three irrigations depending on days to maturity of the variety should be ensured to raise good crop
- Rogueing should be done at three stages viz., before flowering, flowering and maturity
- Required plant protection measures should be adopted to raise healthy crop
- Border row plants of 1m area from all side of plot should first be harvested separately and harvesting should be done at the stage when 70-80% plants turn yellow. The harvested crop should be dried before threshing.
- Oil content and other quality traits need to be analysed before grading by taking random samples from dried seed lot
- The moisture of the seed lot, after threshing, should be dried to 8% moisture level
- Germination test of the individual seed lot need to be done. The seed should be graded before packing
- The graded seed should be treated with Apron 35SD@6g/kg seed or with Thiram@2g/kg to impart protection against diseases during seedling emergence
- The seed should be packed in bags sufficient for minimum standard unit of cultivated land eg. acre, hectare etc.
- Seed at 8% moisture level should be stored at 30% relative humidity and seed store should be properly fumigated to avoid storage pests

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# SEED PRODUCTION TECHNOLOGY OF COTTON

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## Introduction

Cotton accounts for about 75% of total production of the natural fibres. There are 4 cultivated species, viz. *Gossypium hirsutum* and *G. barbadense* (tetraploids) and *G. arboretum* and *G. herbaceum* (diploids). All these 4 species are cultivated in India. Amongst these 4 cultivated species, G*ossypium hirsutum* occupies largest area and accounts for about 90% of total cotton production in India as well as globally. During 2016-17 total cotton production in world was about 22.85 MT from an area of about 28.24 millon ha. Cotton productivity in India has been very low as compared to the major cotton producing countries of the world and was also lower than the world average of about 750 kg lint /ha.

India was the first country in the world to develop hybrid cotton. The first *hirsutum x hirsutum* hybrid 'H-4' was released for commercial cultivation in 1970 by Dr. C.T. Patel at Surat for Gujarat. This was followed by release of inter specific (*hirsutum x barbadense*) hybrid 'Varalaxmi' in 1971. A large number of intra specific and inter specific hybrids have since been developed and commercialized in different parts of the country and occupied about 50% of total area under cotton before the introduction of Bt cotton hybrids. Cotton is an often cross pollinated crop. The average out crossing is about 4-6%. The pollen is heavy and sticky and hence cross pollination occurs only through insects i.e. honey bees and bumble bees. Maintaining genetic purity of parents is very important for hybrid seed production and to ensure availability of quality hybrid seed to the farmer.

## Hybrid Seed Production through hand emasculation and pollination:

Doak (1934) gave thumbnail method of emasculation. Hand emasculation is normally done in the afternoon depending upon the environmental conditions, especially temperature. Mature flower buds, which are most likely to open into a flower next day in the morning, are selected for emasculation. The method involves removal of corolla along with anther sheath by giving shallow cut at the base of the bud with thumbnail and removing corolla and anther column slowly by twisting action. Emasculated flower buds are generally covered with tissue paper bag (9 cm x 7 cm) to prevent contamination from foreign pollen. Pollination is done in the morning next day. Time to start pollination will depend on availability of pollen in the male parent. Pollen from one flower is enough to pollinate 4-5 buds. Though commonly adopted, this method is not much suitable for developing diploid or desi hybrids since the flower buds of these are small and the style is short and brittle making it more difficult for large scale production of hybrid seeds. Nonetheless, this is the most successful

method used in hybrid seed production of tetraploid cotton wherein 40 to 50% or more seed setting is obtained. Surat Method and Straw tube Method of emasculation are also used in cotton.

Hand emasculation and pollination is the conventional method of hybrid seed production in cotton. Majority of the hybrids released so far by the public sector are conventional ones. On the other hand all the Bt cotton hybrids have been developed by the different seed companies in the private sector. The conventional method involving hand emasculation and pollination is being used for hybrid seed production. The following factors are important for good quality hybrid seed production.

**(i). Genetic Purity of Parental Lines:**

Proper site selection, medium to heavy, deep and well drained soil is considered ideal for seed production. Nucleus seed of parental lines is produced by the breeder. The isolation distance for nucleus seed production is 30m. Nucleus seed is used for production of breeder seed of such lines which is further multiplied to produce the required quantity of foundation seed. Strict seed production standards are followed for maintaining genetic purity of parental lines.

**(ii). Isolation Distance in Seed Production Plot:**

The cotton hybrid seed production plot should have 30 m isolation distance on all sides from other fields. The isolation distance between parents should be at least 5 m.

**(iii). Agronomy and plant protection:**

The female parent may be planted at wide spacing of 90cm x 90cm or 90cm x 60cm for easy mobility. Fertilizer dose of NPK depending on the soil fertility. Foliar application of 2% DAP also helps in better retention of bolls. Appropriate plant protection measures may also be followed to minimize losses caused by insects. The hybrid seed may preferably be produced under irrigated conditions.

**(iv). Synchrony of Parental lines:**

The parental lines may be of similar duration with regard to flowering and maturity. In case of differences the sowing may be done accordingly to ensure synchrony in flowering. The sowing dates of parental lines are adjusted in such a way that there is synchronization of flowering in female and male parent so that there will be continuous supply of pollen till the crossing season is over. Staggered planting of male parent is generally done depending on the date of flowering in male and female lines.

**(v). Trained manpower**: Qualified technical staff, trained labour, etc. are important for hybrid seed production. One person can emasculate and pollinate about 250-400 flowers/day (FAO standard). Picking is done for only the completely opened bolls. Damaged and under developed bolls should be separated and removed before sorting.

**Factors affecting yield and quality of hybrid seed:**

1. The planting ratio of male and female parents.
2. No of male flowers used in pollinating one female flower.
3. Not following the staggered sowing to synchronize flowering in male and female lines.
4. Stage and period of crossing: Seeds produced after crossing during the second fortnight of flower initiation has the highest germination compared to later produced hybrid seeds.
5. Vigour of female parental line and pollen production ability of male parent.
6. Damage to style and stigma.

**Seed Certification Standards:**

***Isolation Distance:***

| **Contaminants** | ***Minimum distance (meters)*** | |
|---|---|---|
| | ***Foundation*** | ***Certified*** |
| 1 | 2 | 3 |
| Fields of other varieties of the same species including commercial hybrid of the same variety | 50 | 30 |
| Fields of the same variety (code designation) not conforming to varietal purity requirements for certification. | 50 | 30 |
| Fields of other varieties of different spp. (different ploidy levels) | 5 | 5 |
| Between blocks of the parental lines of the same hybrid | - | 5 |

**Minimum Seed Standards for varieties:**

| **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 |
| Weed seeds (maximum) | 5/kg | 10/kg |
| Germination (minimum) | 65% | 65% |
| Moisture (maximum) | 10.0% | 10.0% |
| For vapour-proof containers (maximum) | 6.0% | 6.0% |

**Minimum Seed Standards for hybrids:**

| Hybrid | Conventional Hybrids | Male Sterility based Hybrids |
|---|---|---|
| Physical Purity | 98% | 98% |
| Genetic Purity | 90% | 95% |

| | | |
|---|---|---|
| Selfed Seed | 8.5% | 1.5% |
| Off type Plants | 1.5% | 1% |
| Germination | 65% | 65% |

# SEED PRODUCTION TECHNOLOGY OF PULSE CROPS

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Pulses or dals contain rich sources of protein for human consumption and considered to be wholesome food. In India, a large proportion of the Indian population is vegetarian and pulses form the main source of protein (18–25 per cent) in the daily diet, complementing the staple cereals in the diets with proteins essential amino acids, vitamins and minerals. Besides, they also provide significant nutritional and health benefits, as listed below:

- Low fat / high complex carbohydrate content — Dieting
- Reduction of plasma cholesterol — Cardiovascular disease
- Low glycemic index — Diabetes
- Colonic bacterial fermentation — Bowel health
- Phytochemical content — Anti-cancer agents

Pulses are some of the most ecologically sustainable agricultural solutions for the soil. Pulses are cultivated under on rainfed conditions, i.e. these crops less irrigation as compared to cereal crops. By growing pulses, farmers can actually increase soil fertility. Pulses are leguminous crops; they have a natural ability to fix atmospheric nitrogen in the soil. Legume plants have a symbiotic relationship with certain bacteria known as *Rhizobia* that live in their root nodules. These bacteria take nitrogen, an essential plant nutrient, from the air and convert it to a form that can be used by plants. Pulses can be grown on range of soil and climatic conditions and play important role in crop rotation, mixed and inter-cropping, maintaining soil fertility through nitrogen fixation, release of soil-bound phosphorus, and thus contribute significantly to sustainability of the farming systems.

The benefits from adopting pulses as a rotational crop include:

- An increased supply of soil nitrogen through nitrogen fixation approximately 40kg / ha of nitrogen
- Agronomic benefits for the succeeding crop — better crop quality (for instance, protein premium in wheat) and improved yield
- The cultivation of pulses has several benefits for the Indian farming community and society in general:
- Reduction of fertiliser costs, as farmers do not need to use nitrogen-based inputs
- Natural rejuvenation of the soil, as pulse crops fix nitrogen
- Low water usage, as the crops do not require extensive irrigation
- Reduction in $CO_2$ emissions, as there is a reduced need for fertilizers and subsequently, fossil fuels that are used to manufacture fertilizers

- Reduction in greenhouse gases (along with the absorption of CO2, pulse crops also lower nitrous oxide levels in the atmosphere by their ability to fix nitrogen)
- Providing an vegetarian alternative to animal protein

The Food and Agriculture Organization (FAO) of United Nations defines pulses as an annual leguminous crop yielding from one to twelve seeds within the pod and harvested for dried seeds. According to FAO classification, there are 11 primary pulses/pulse groups. They include (i) dry beans which cover kidney bean, lima bean, adzuki bean, mung bean, urad bean, scarlet runner bean, moth bean, leper bean, (ii) dry broad beans consisting of horse bean, broad bean, field bean, (iii) dry peas covering garden pea, protein pea, (iv) chickpea/ Bengal gram /chana/gram, (v) pigeon pea/tur/ arhar, (vi) lentil/ masur, (vii) dry cowpea, ( viii) earth pea, (ix) vetch, (x) lupines and (xi) minor pulses like lablab, hyacinth bean, jack bean, winged bean, velvet bean, yam bean.
Pulses are grown in an area of 73.33 million ha with a production of 61.34 million tons in World. India is the largest producer, consumer and importer of pulses in the World, representing 25 per cent of total production, 30 per cent of total consumption and 33 per cent of global acreage under pulses. However, the productivity of pulses in India has been very low at 622 kg/ ha, as compared to1908 kg/ha in Canada / USA; mainly due to he vagaries of monsoon, problem of quality and approved HYV seeds, low seed replacement rate, etc. Hence, it imports a large amount of pulses to meet the growing domestic needs. During 2009-10, India imported 3.5 million tons of pulses from the countries like Australia, Canada, and Myanmar. On the other hand, India is also the largest pulses processor, as pulses exporting countries like Myanmar, Canada and Australia do not have adequate pulses processing facilities
Major pulses grown in India include chickpea or Bengal gram (*Cicer arietinum*), pigeonpea or red gram (*Cajanus cajan*), lentil (*Lens culinaris*), urdbean or black gram (*Vigna mungo*), mungbean or green gram (*Vigna radiata*), lablab bean (*Lablab purpureus*), moth bean (*Vigna aconitifolia*), horse gram (*Dolichos uniflorus*), pea (*Pisum sativum* var. arvense), grass pea or khesari (*Lathyrus sativus*), cowpea (*Vigna unguiculata*), and broad bean or faba bean (*Vicia faba*). More popular among these are chickpea, pigeonpea, mungbean, urdbean and lentil. In general, pulses are mostly grown in two seasons: (i) the warmer, rainy season or kharif (June-October), and (ii) the cool, dry season or rabi (October-April). Chickpea, lentil, and dry peas are grown in the rabi season, while pigeonpea, urdbean, mungbean, and cowpea are grown during the kharif season. As per IIPR Vision 2030, chickpea dominates with over 40 percent share of total pulse production among various pulse crops, followed by pigeonpea (18-20%), mungbean (11%), urdbean (10-12%), lentil (8-9%) and other legumes (20%). MadhyaPradesh, Maharashtra, Uttar Pradesh, Andhra

Pradesh, Karnataka and Rajasthan are the major states growing pulses in India. These six states contribute 80% of total pulse production and area.

**Table 1:** List of important pulse crops

| S. No. | Crop | Botanical name |
|---|---|---|
| 1. | Black Gram | *Vigna mungo* (L.)Hepper |
| 2. | Bengal gram | *Cicer arietinum* (L.) |
| 3. | Cowpea [Asparagus bean, Yard long bean] | *Vigna unguiculata* [L.] Walp) syn. *V. sinensis* [L.] Savi. ex Hassk., *V. catjang* [Burm. f] Walp., *V. cylindrical* [L.] Skeels., *V. sesquipedalis* Fruhw.] |
| 4. | Green gram | *Vigna radiata* (L.) Wilczek |
| 5. | Horse gram | *Macrotyloma uniflorum* (Lam.) Verdc.) |
| 6. | Indian bean | *Lab lab purpureus* (L.) Sweet |
| 7. | *Khesari* (Chickling vetch) | *Lathyrus sativus* L. |
| 8. | Lentil | *Lens culinaris* Medic. |
| 9. | Moth bean (Kidney bean) | *Vigna aconitifolia* (Jacq.) Marechal |
| 10. | Peas (Field pea and Garden pea) | *Pisum sativum* var. *arvense* (L.) Poir) |
| 11. | Pigeonpea (*Arhar*) | *Cajanus cajan* (L.) Millsp. |
| 12. | *Rajmash* (French bean) | *Phaseolus vulgaris* L. |

**Table2:** Varieties of pulse crops released by ICAR-IARI, New Delhi

| S. No. | Name of the Variety | Year of Release | States for which Released | Characteristics |
|---|---|---|---|---|
| **CHICKPEA** | | | | |
| **1.** | Pusa 2085 | 2013 (SVRC) | National Capital Region, Delhi | Yield: 20q/ha under irrigated conditions. It is a large seeded kabuli chickpea variety with 100 seed weight of 36 g. It has multiple disease resistance against dry root rot and stunt; moderately resistant to wilt and botrytis gray mold and tolerant to collar rot. The seeds are beige coloured, uniform, attractive and shinning. |
| **2.** | Pusa Green 112 | 2013 | National Capital Region, Delhi | Yield: Average yield is 23 q/ha and has a yield potential of 27 q/ha. |

| | | | | |
|---|---|---|---|---|
| | | | | It is a high yielding desi green seeded chickpea variety having high resistance to Fusarium wilt and drought. Seeds are dark green, uniform and excellent for cooking and culinary purpose. It will be boon for marginal farmers as it has multiple stress resistance. Green seeded chickpeas are in great demand in urban areas for culinary and table purposes. |
| **3.** | Pusa 5028 | 2011 (SVRC) | National Capital Region, Delhi | Yield: 27 q/ha under irrigated conditions. An extra bold (41 g/100 seed) desi chickpea variety moderately resistant to wilt. |
| **4.** | Pusa 5023 | 2011 (SVRC) | National Capital Region, Delhi | Yield: 25 q/ha under irrigated conditions. An extra bold (50 g/100 seed) kabuli chickpea variety moderately resistant to wilt. |
| **5.** | Pusa 2024 (Kabuli) | 2008 (SVRC) | National Capital Region of Delhi | Yield: 2.5-2.8t/ha Moderately resistant against soil borne diseases and drought. It matures in 145 days. |
| **6.** | Pusa-2024 (Kabuli) | 2008 | National Capital Region of Delhi | Yield: 30.0 q/ha Moderately resistant against soil borne diseases and pod borer. |
| **7.** | BGD 128 (Kabuli) | 2007 | Central India (MP, Maharastra, Gujrat, Bundelkhand parts of UP and adjoining parts of Rajasthan) | Yield: 20.0 q/ha Resistance against soil borne diseases and wide adaptation. Due to its semi-erect growth habit, It is suitable for mechanical harvesting. |
| **8.** | Pusa 1108 (Kabuli) | 2006 (SVRC) | National Capital Region of | Yield: 2.5-3.0t/ha Resistant to soil borne diseases. Having bold, uniform, creamy |

| | | | | |
|---|---|---|---|---|
| | | | Delhi | coloured, attractive grains with excellent cooking quality, the variety fetches high market prices. It matures in 145-150 days. |
| **9.** | Pusa 547 (Desi) | 2006 (CVRC) | Delhi, Haryana, Punjab, U.P. and Rajasthan | Yield: 1.8-2.5t/ha<br>It is of medium maturity (135 days), tolerant to wilt, root rot and stunt diseases. |
| **10.** | Pusa Subhra (BGD 128) | 2006 (CVRC) | M.P., Maharashtra, Gujarat, Bundelkhand parts of U.P. and adjoining parts of Rajasthan | Yield:1.7-2.3t/ha<br>Moderately resistant to soil borne diseases and stunt. Owing to its semi-erect growth habit, it is suitable for mechanical harvesting. It matures in 110-115 days. |
| **11.** | Pusa 1105 (Kabuli) | 2005 (SVRC) | National Capital Region of Delhi and Karnataka | Yield:2.5-3.0t/ha<br>Bold seeded (30 g/100 seeds), moderately resistant against soil borne diseases and highly tolerant against drought. It matures in 145 days in north India and 120 days in south India. |
| **12.** | Pusa 1103 (Desi) view image | 2005 (SVRC) | National Capital Region of Delhi | Yield:2.0-2.4t/ha<br>First variety developed by using a wild species (C. reticulatum). It possesses resistant against wilt/root rot, drought and high temperature. Suitable for rice based cropping system in northern India. It matures in 130-140 days. |
| **13.** | Pusa 1088 (Kabuli) | 2004 (SVRC) | National Capital Region of Delhi | Yield:2.0-3.0t/ha<br>It is resistant to Fusarium wilt, root rot and stunt virus. Bold seeded and matures in 135-140 days. |
| **14.** | Pusa chamatka | 1999 (CVRC) | Delhi, Haryana, | Yield:2.5-3.0t/ha<br>Resistant to soil borne diseases; |

| | | | | |
|---|---|---|---|---|
| | r (BG 1053) (Kabuli) | | Punjab, Rajasthan and U.P. | bold seeded; good cooking quality. It matures in 145-450 days. |
| **15.** | Pusa Dharwar Pragati (BGD 72) | 1999 (CVRC) | M.P, Maharashtra, Gujarat, Bundelkhand, parts of U.P. and adjoining parts of Rajasthan | Yield:2.2-2.8t/ha<br>Moderately resistant to soil borne diseases and resistant to drought. Bold seeded and matures in 115-120 days. |
| **16.** | Pusa 362 (Desi) | 1994 (CVRC) | North India | Yield:2.5-3.0t/ha<br>It is resistant to soil bore diseases, tolerant to drought and very good for cooking; Grains and very good with yellowish brown colour. It matures in 155 days. |
| **17.** | Pusa 372 (Desi) | 1993 (CVRC) | Delhi, Haryana, Punjab, Rajasthan, U.P., Bihar, M.P., Gujarat and Maharashtra | Yield : Late sown - 1.8-2.2t/ha<br>Normal-2.5-3.0t/ha<br>Resistant to soil bore diseases; good for dal and besan making; very wide adaptability and drought tolerant. It matures in 140-145 days. |
| **PIGEON PEA** | | | | |
| **18.** | Pusa 2002 | 2008 (SVRC) | National Capital Region of Delhi | Yield: 1.77t/ha; This variety can fit well in the double cropping system. A timely sown crop can be harvested by the second week of November, thus leaving the field for rabi crops. It matures in 40-145 days. |
| **19.** | Pusa 2001 | 2006 (SVRC) | National Capital Region of Delhi | Yield: 2.0t/ha; High yielding variety and suitable for arhar-wheat rotation. It matures in 140-145 days. |
| **20.** | Pusa 991 | 2005 (SVRC) | National Capital Region of Delhi | Yield: 1.84t/ha; Highly suitable for rainfed and salinity prone areas. Seed is medium bold (7.9 g/ 100 seeds), brown, shining |

| | | | | |
|---|---|---|---|---|
| | | | | and round. It matures in 140-145 days. Suited for arhar-wheat rotation. |
| **21.** | Pusa 992 | 2005 (CVRC) | Haryana, Punjab, Rajasthan, Western Uttar Pradesh and Delhi | Yield: 1.65t/ha; Medium bold seed (8.5g/100 seeds), brown, shining and round. Suited for arhar- wheat rotation. It matures in 140-145 days. |
| **22.** | Pusa 9 | 1993 (CVRC) | North-eastern, eastern and central regions | Yield: 2.0-2.5t/ha; Medium height, suitable for intensive farming, resistant to Alternaria blight disease. It matures in 240 days. |
| **MUNG BEAN** | | | | |
| **23.** | Pusa 0672 | 2009 (CVRC) | Northern hilly region | Yield: 0.95-1.0t/ha Tolerant to yellow mosaic virus. Seeds are of medium size, attractive and green in colour, It matures in 52-103 days. |
| **24.** | Pusa Ratna | 2005 (SVRC) | National Capital Region of Delhi | Yield: 1.2t/ha It matures in 65-70 days and synchronous in maturity. Tolerant to yellow mosaic virus. |
| **25.** | Pusa Vishal | 2001 (CVRC) | Western Uttar Pradesh, Haryana, Punjab, Rajasthan, plains of Himachal Pradesh and J&K | Yield: 1.2t/ha Resistant to mungbean yellow mosaic virus. It matures in 65-70 days in spring and 60-65 days in summer seasons and synchronous in maturity. |
| **26.** | Pusa 9531 | 2001 (CVRC) | Punjab, Haryana, western Uttar Pradesh, Rajasthan, plains of J & K and Himachal Pradesh | Yield: 1.2t/ha It matures in 60-65 days. Pods are light brown in colour at maturity and tolerant to yellow mosaic virus. |

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| **LENTIL** | | | | |
| **27.** | Pusa Lentil 5 (L 4594) | 2006 (SVRC) | National Capital Region of Delhi | Yield: 1.7t/ha<br>It has medium growth habit, small seeded, orange cotyledon and resistant to rust. It matures in 125-135 days. |
| **28.** | Pusa Vaibhav (L 4147) | 1997 (CVRC) | Western Uttar Pradesh, Haryana, Punjab, Rajasthan, Uttarakhand, parts of Rajasthan and Himachal Pradesh | Yield: 1.7t/ha<br>The seeds are small with orange cotyledons. It is resistant to wilt and rust and matures in 120-125 days. |
| **29.** | Shivalik (L 4076) | 1995 (CVRC) | Western Uttar Pradesh, Haryana, Punjab, Uttarakhand, parts of Rajasthan and Himachal Pradesh | Yield: 1.5t/ha<br>It is resistant to wilt and rust and matures in 120-15-25 days. |
| **FIELDPEA** | | | | |
| **30.** | Pusa Mukta (DDR 55) | 2005 (SVRC) | National Capital Region of Delhi | Yield: 2.5t/ha<br>A bold seeded variety having resistant to powdery mildew. It matures in 110-115 days. |
| **31.** | Pusa Panna (DDR 27) | 2001 (CVRC) | Western Uttar Pradesh, Haryana, Punjab, Rajasthan and Uttarakhand | Yield: 1.7t/ha<br>It is a dwarf, extra-early (90 days) and powdery mildew resistant variety. |
| **32.** | Pusa Prabhat (DDR 23) | 2001 (CVRC) | U.P., Bihar, West Bengal and Assam | Yield:1.5t/ha<br>It is a dwarf, extra maturing (average 102 days) and powdery mildew resistant v |
| **COW PEA** | | | | |

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| **33.** | Pusa 578 | 2005 (SVRC) | National Capital Region of Delhi | Yield: 1.2t/ha<br>It is resistant to yellow mosaic virus. It matures in about 90 days. |
| **34.** | Pusa Sampada (V 585) | 1999 (CVRC) | Plains | Yield: 0.86t/ha<br>It is resistant to yellow mosaic virus. It matures in about 100 days. |

**Potential to improve the productivity of pulse crops:**

By adopting some of the best practices across the world, India has the potential to increase its average yield to about 1200kg / ha. India can increase production through several routes,

- Increasing total acreage under pulses — by converting rice fallow lands, adopting intercropping, using short duration seeds, etc
- Improving yields for pulses — by using new varieties of seeds such as high yield, hybrid, treated, pest and disease-resistant, and through proper crop protection, pest management, crop nutrition, etc
- Reducing post harvest losses — by treating for storage pests, increasing the pulse- processing infrastructure, building an efficient supply-chain mechanism, etc

There are certain seed technological constraints, which restricts the production and availability of quality seed to the farmers:

- Proper plant population establishment
- Screen size recommended for processing of pulses do not hold appropriate for all the varieties of the same crop.
- Rejection of seed plots by certification agencies on doubtful characters.
- Certification regulations imposed by certification agencies.
- Un-identical seed certification standards for field and seed.
- Seed losses during storage caused by deterioration in viability and vigour and insect pest damage.
- Delayed planting of seed crop resulting in production of undersized seed with rudimentary/premature embryo.
- Increased frequency of hard seeds.
- Non-existence of long/medium term storage facilities.

**General Principles of Seed Production**

- The varieties have to be grown in their recommended areas of adaptation only.
- Use of seeds from approved/ authentic source and of designated class for seed multiplication.

- The seed crop on should be grown on seed production plots, which meet the preceding crop requirements.
- Recommended isolation of seed crops should be followed in order to prevent genetic contamination throughout crossing and to avoid physical admixtures.
- Standardized crop management practices to ensure better seed quality and abundant seed yields.
- Rouging of seed production plots to ensure the removal of undesirable/ off-type plants prior to the stage at which they could cause contamination.
- Application of prophylactic control measures to ensure the production of disease free seeds.
- Harvesting of the seed crop at appropriate physiological maturity stage for the production of highly vigorous and viable seeds.
- Use of appropriate seed harvesting and threshing methods, which cause no/ minimal injury to the seeds.
- Up-gradation of seed quality through processing and grading.
- Seed quality testing in the laboratory for moisture, germination purity and health components.
- Seed treatment with appropriate fungicides/insecticides to prevent inoculum/ infestation.
- Packaging, labeling and sealing of the seeds to ensure the delivery of the quality seed produced through a rigorous system of quality control to the user.

**Precautions to be taken in seed production:**

- Procurement of seed from authentic/approved source.
- The receipt should be obtained upon purchase of seed from seed seller/ dealer.
- The tags & bags should be kept for registration.
- Implements (Seed drill, Harvester, Winnower etc.) should be cleaned after use of one variety two another.
- The rogues and off-type plants should not be allowed in the seed production plots.
- Appropriate control measures should be adapted against pest and diseases.
- Recommended isolation distance should be maintained.
- The seed should the packed in clean/new bags.
- The seed should be stored after drying to safe moisture content.

These techniques contribute for higher seed yield and better seed quality. Any deficiencies in the application of these measures may cause deterioration of the varieties during production cycles.

The seed production technology of important pulse crops is discussed below:

## 1. Green gram and black gram *(Vigna radiata* and *Vigna mungo)*

The optimum season for seed production is June – August, September – November and February – March for both black gram and green gram. Though it can be grown in all the three seasons, Spring –summer is better suited for seed production programmes.

**Method of seed production:** Green gram and black gram are highly self pollinated crops with cross pollination to the extent of 5 – 10%. The crop should be raised in isolation and seeds are allowed to set by self pollination. To maintain the varietal purity an isolation distance of 5 metres for certified and 10 metres for foundation seed production is maintained between varieties and of the same variety not conforming to varietal purity requirements of certification.

**Land selection:** The land selected should be free from volunteer plants. The soil should be fertile with neutral pH and proper drainage facility. It should be prepared to fine tilth. Ridges are formed with a gap of 60 – 90 cm.

**Seed selection and sowing:** The variety selected should be recommended for the season/region. The seed should be procured from an authentic source/ authorized dealer. The seed should be of good quality (healthy with a good germination percentage and labeled), graded and labeled. Remove the off colour and out sized seeds. Seed rate is 8 kg/ acre (20 kg/ha).
Seed treatment with Thiram/Carbendazim/ Captan @ 0.25% and with Gaucho/ Cruiser (Thiamathaxom) @ 5ml/kg of seed against MYMV. Seed treatment with recommended Rhizobium strain (200 gm/10 kg of seed Selected seeds should be treated with *Trichoderma viride* @ 4 gm/kg of seeds or *Pseudomonas* @ 10 gm/kg of seeds. Mix *Trichoderma* or *Pseudomonas* in rice gruel and mix the solution with seeds. Shade dry the seeds for 30 minutes before sowing. Treating the seeds with *Trichoderma* or *Pseudomonas* protects the crops from disease causing microorganisms. Seeds should be treated with *Rhizobium* @ 250 gm/acre (600 gms / ha) to facilitate natural nitrogen fixation by plants. Treated seeds should be dibbled at 3-4 cm depth at the side of the ridges. Sowing should be planned in such a way that the maturation of seeds does not coincide with rains. It will increase the percentage of off-coloured seeds. The spacing maintained is 25 x 10cm for rainfed and 30 x 10cm for irrigated crop.

**Nutrient management:** During land preparation, farmyard manure @ 10 truck loads/acre (25 truck loads/ha) should be added and incorporated into the soil by ploughing. NPK @ 10:20:10 kg/acre (25:50:25 kg/ ha) is

recommended for irrigated crop, whereas for rainfed crop it is 5:10:5 kg/acre (12.5:25:12.5 kg/ha) for good seed yield. This will increase the number flowers and improve the seed setting and quality of the seeds.

**Weed management:** Hand weeding is done on 15th and 30th day after sowing.

**Pest and disease management:** Aphids, army worms, pod borer, white fly, Mungbean yellow mosaic virus (MYMV), leaf curl and leaf crinkle (Virus), root rot and powdery mildew are the common pests and diseases that affects the green gram and black gram crop. The management measures for these pest and diseases.

**Irrigation:** The first irrigation is done soon after the sowing and life irrigation is done on the third day. After this, irrigation should be done once in 10 – 15 days depending upon the soil and climatic conditions. Water stagnation should be avoided at all growth stages. Irrigation during flowering and pod formation stages are very critical. Irrigation should be terminated at 50 DAS so that 60-70% pods are mature.

**Maintaining seed quality by thorough roguing:** One of the most important aspects of seed production is thorough rouging. Rogue is defined as the presence of those plants that deviate from the characters described for the variety. Such rogues if left in the field they tend to reduce purchase value of resultant seed. Roguing is defined as the operation of removing rogues. In practice, all plants that do not obey the characteristics of the particular seed crop are to be removed along with diseased plants, other crop plants, weeds, insect affected plants during roguing operation.

**When to do rouging?** Roguing is more effective if done prior to flowering, since upon flowering there is very high possibility of cross pollination. Though green gram is self pollinated, some extent of cross pollination is caused by bees. Roguing is also attempted during flowering, pod formation stage and prior to harvest in case of pulse crop.

**Vegetative phase:** During vegetative phase i.e., during first 25 days, rouging is attempted based on plant characters like height of plant, leaf shape, size, venation and presence or absence of hairs on plant surface. Plants showing tendril formation and those showing symptoms of root rot, wilt and yellow mosaic are also removed.

**Flowering stage:** The rogues are identified based on flower characteristics. During this phase when most of the plants are flowering, all those plants still in

vegetative phase are also removed and those that flower too early also to be removed.

**Pod formation stage:** The rogues are removed based on pod characteristics like length of pod, pod width, shape of tip, colour of pod (green, red, blotched).

**Pod formation stage:** The rogues are removed based on pod characteristics like length of pod, size, shape, colour of suture, presence or absence of bend at pod tip.

**Pod harvest stage:** Roguing is done prior to harvest based on seed characteristics like colour of seed, luster and size of seed.

**Precautions-** All the rogues must be immediately removed from the seed field and destroyed. Maximum percentage of offtypes permitted at final inspection is 0.10% for foundation seed production and 0.20% for certified seed production. In case of green gram, the permitted limits of seed borne disease like halo blight affected plants is 0.10% for foundation and 0.20% for certified seed production.

**Field inspection:** A minimum of two inspections will be done from flowering to maturity stage by the Seed Certification Officer. The first inspection is done during flowering followed by second in flowering and maturity stage to check the isolation, off- types, volunteer plants, diseased plants and estimated yield.

**Harvesting:** Harvest is done soon after the maturation of the seeds. Seeds attain physiological maturity 30 days after 50% flowering. The mature pods of black gram turn black and greengram pods turns brown. At this stage the moisture content of the pods will be 17 – 18%.

**Proper care during harvest:** The seed crop comes to harvest 55-65 days after sowing and it varies according to the varieties, this is generally 30 days from 50% flowering. At this time, the pods turn brown to black from green. Prior to harvest there is an important pest to be controlled; Bruchids are the major pests of stored pulse seeds.

**Pre-harvest sanitation spray:** Bruchids lay eggs on the surface; the grubs bore into the seeds and eat the cotyledon. Those seeds store poorly and loose their viability faster. It has been found that bruchids lay their eggs on the pods while in the field itself. Hence control of these pests must start from the field itself. The field carry-over of Bruchids can be controlled if the crop is sprayed using Quinalphos 25% EC (2 ml of insecticide per litre) ten days prior to harvest.

**Threshing and processing:** Harvested pods along with plants are dried to a moisture content of 12 – 13% and then threshed using sticks. Threshed grains are cleaned and dried to attain a moisture content of 8 – 9%. The seeds are graded using BSS 7 x 7 wire mesh sieve. Alternatively, the pods may be picked and seeds from first two pickings may be retained for seed purpose.

**Drying and storage;** Processed and graded grains are further dried to attain 9% of moisture content. Then seeds should be mixed with 3% neem seed kernel power to preserve the seeds from storage pests especially infestations of the bruchid beetle.

2. **Chickpea:**

**Crop season and sowing time:** Chickpea is grown in *rabi* (postrainy season) following a *kharif* (rainy season) crop or *kharif* fallow. The sowing is done in the month of October or November. Late sowing (December-January) should be avoided as the late-sown crop may experience moisture stress and high temperatures at the critical stage of pod-filling, leading to reduced yield and seed quality.

**Isolation distance:** Isolation of a seed crop is done by maintaining a distance from other nearby fi elds of the same crop and other contaminating crops. Chickpea being a self-fertilized crop has a very low outcrossing percentage (0-1%). In India, an isolation distance of 10 m for foundation seed and 5m for certified seed is required.

**Suitable soil type:** Chickpea can be successfully grown in a variety of soil types including coarse-textured sandy to fi ne-textured deep black soils (vertisols). However, the best suited soils are deep loams or silty clay loams with a pH ranging from 6.0 to 8.0. Saline soil and fields with a high water table are not suitable for chickpea.

**Field preparation:** Chickpea plants are highly sensitive to poor aeration in the soil. Seedling emergence and plant growth are hindered if field surface is compact. Therefore, the field should have loose tilth and good drainage. The stubble and debris from the previous crop should be removed as these can harbor the pathogens that cause root diseases, such as collar rot.

**Sowing:** Sowing is usually done on conserved soil moisture. A pre-sowing irrigation may be needed, if the available soil moisture is not adequate for germination. Kabuli chickpea should never be irrigated immediately after sowing, particularly in deep black soils. This is because the kabuli chickpea

seeds have thin seed coat and deteriorate faster as compared to desi type and are also more susceptible to seed rot and seedling damping off.

**Sowing depth:** Seed should be sown deeply enough to make contact with moist soil. A depth of 5-8 cm seems to be ideal for the emergence of chickpea.

**Spacing:** Line sowing is a must in the crop grown for seed production as it facilitates interculture operations, rouging and field inspection. Row-to-row spacing of 30 cm and plant-to-plant spacing of 10 cm are generally used, which give a plant population of about 33 plants per m2 (330,000 plants ha-1). Wider row spacing (45–60 cm) can be used in large seeded kabuli chickpea and irrigated crops (both desi and kabuli types), which are expected to have greater plant width. Broadbed and furrow system or ridge and furrow system are very useful for irrigation, drainage and interculture operations and are widely used at ICRISAT.

**Seed rate:** It differs from variety to variety, depending on seed size. For initial seed multiplication of a new variety, the multiplication rate (yield per plant) is more important than yield per unit area. The following guidelines may be used for seed rate:

**Seed treatment:** The seeds should be treated with fungicides (2g thiram +1g carbendazim kg-1 seed) before sowing for reducing seed and soil borne fungal diseases. Phosphorus solubilizing bacteria (PSB) have been identified, which improve availability of phosphorus to plants. Thus, seed treatment with PSB is recommended. If chickpea is being grown for the fi rst time, the seeds should be inoculated with *Rhizobium* culture. The seeds should be treated first with fungicides and then with PSB and *Rhizobium,* following the procedure recommended by suppliers. The culture-treated seeds should be dried in the shade and sown as soon as possible thereafter. If the seed is to be treated with pesticides, always apply insecticides first, followed by fungicides, and finally *Rhizobium* culture/phosphate solubilizing bacteria or follow instructions on the packets.

**Fertilizer application:** Fertilizer requirements depend on the nutrient status of the field, and thus, vary from field to field. Therefore, the doses of fertilizers should be determined based on the results of soil test. The generally recommended doses for chickpea include 20–30 kg nitrogen (N) and 40–60 kg phosphorus (P) ha-1. If soils are low in potassium (K), an application of 17 to 25 kg K ha-1 is recommended. There will be no response to application of K in soils with high levels of available K. Total quantities of N, P and K should be given as a basal dose. Foliar spray of 2% urea at fl owering has been found beneficial in rainfed crops.

**Micronutrients:** Intensive cropping without application of micronutrients, limited or no application of organic fertilizers and leaching losses lead to deficiency of one or more micronutrients in the soil. The important micronutrients for chickpea include sulphur (S), zinc (Zn), iron (Fe), boron (B) and molybdenum (Mo). The requirements of these micronutrients vary from field to field and should be determined based on the results of soil analysis.

**Irrigation:** Chickpea is generally grown as a rainfed crop, but two irrigations, one each at branching and pod filling stages, are recommended for higher yield. Higher number of irrigations may lead to excessive vegetative growth in heavy soils.

**Weed management:** Chickpea is a poor competitor with weeds at all stages of growth. Pre-emergence herbicides, such as BASALIN (Fluchloralin) @ 1.5-2.0 l $ha^{-1}$ or STOMP (Pendimethalin) @ 3.0 l $ha^{-1}$ were found effective in controlling early flush of weeds. Mechanical and/or manual weeding can be done where wide row spacing is used.

**Plant protection:** Chickpea being a rich source of protein is prone to damage by insect-pests and diseases. In general, root diseases (fusarium wilt, collar rot and dry root rot) are more prevalent in central and peninsular India, whereas foliar diseases (ascochyta blight, botrytis gray mold) are important in northern, north-western and eastern India. Among the insect pests, pod borer (*Helicoverpa armigera*) is the most severe yield reducer throughout India in the field, while bruchids (*Callosobruchus chinesis*) cause severe damage in storage. For control of pod borer, application of chemical sprays (endosulfan @ 350 g a.i. ml $ha^{-1}$, Indoxacarb @ 70 ml a.i. $ha^{-1}$ (TRACER-200 ml) or Spinosad @ 45 ml a.i. $ha^{-1}$ (AVAUNT @ 400-500ml) can be applied as and when needed.

**Roguing:** It refers to systematic examination of seed production fi elds and removal of undesirable plants that may contaminate the seed crop. Roguing not only maintains varietal purity but also protects the seed crop from seed-borne diseases. The off-type plants, other crop species (with similar seed size), weed plants, parasitic weeds such as *Cuscuta* spp. and plants infected with seed-borne fungal diseases and viruses should be removed from the seed fields from time to time.

**Harvesting and threshing:** The time of harvesting is crucial in maintaining the quality of seeds. The crop should be harvested when leaves start to senesce and start shedding, pods turn yellow, plants are dry, and seed feels hard and rattles within the pod. After harvest, the plants can be dried in the sun for a few days to ensure that seeds get dried well. Threshing can be done using commercially available power threshers.

**Seed processing:** The dried seeds are cleaned to remove the undesirable contaminants such as plant parts, soil particles, stones, weed seed, other crop seed, and shriveled, broken, or damaged seed. Cleaning and upgrading is based on physical differences between good seed, poor seed and undesirable contaminants. The cleaning and grading of seeds is first achieved by winnowing and then through a set of mechanical sieves. In addition to air cleaners and aspirators, indented separators, disc separators, gravity separators, spiral separators and drum separators are frequently used.

**Seed storage:** The seed must be properly dried before storage. The ideal seed moisture level is 10-12% for short-term storage (up to 8 months). After drying, the seed should be either stored in polythene-lined gunny bags or in safe storage structures (metal bins or earthen containers). The bags should be kept in a rodent free room and placed on wooden planks (not more than fi ve in a stack) and away from walls to avoid dampness to the seeds. Bruchids (*Callosobruchus* spp.) are the most serious storage pests of chickpea and all other food legumes. Bruchids lay white eggs on the seeds and the larvae bore into seeds and adults emerge from the seeds by cutting round holes in the seeds. The infested seeds are unfit for sowing and consumption. Proper control measures should be undertaken to protect chickpea seed from bruchids. The traditional methods of protecting the seed from bruchid damage by mixing with ash, dried neem leaves, or chickpea or wheat straw are useful for small quantities of seed. In case of large scale storage, the seed store or the seed bins should be fumigated periodically with commercially available fumigants (ethylene dibromide or phosphine) to protect seed from storage pests. The main advantage of fumigation is that all stages of the insect, including eggs, larvae and pupae, are controlled and also affect other storage pests and rodents.

## 3. Pigeon pea

The major pigeon pea growing states are Maharashtra, Karnataka, Uttar Pradesh., Andhra Pradesh, Gujarat and Madhya Pradesh.

**Origin and Distribution:** It is considered to be of Indian origin and distribution. It was introduced in the African continent about 1000 years ago. Indian varieties have been grossly divided in two chief types:

- *Cajanus cajan* var. *flavus* (tur): Includes short-duration annuals having smaller plants, usually yellow flowers and fewer plain pods. These are mainly grown in southern India
- *Cajanus cajan* var. *bicolor* (arhar): This includes long-duration, late-maturing types having tall growth and bushy habit. Flowers are yellow with purple streak. Pods are dark coloured with 4-5 seeds. These are more prevalent in northern India

**Climatic requirement:** Pigeon-pea is highly drought resistant crop. It is mostly grown as rainfed crop in dryland areas but also grown under irrigated conditions in UP, Haryana, Gujarat, Maharashtra and Karnataka. It can successfully grow in areas receiving only 65 cm annual rainfall as the crop matures fast and pest attack is low. It is photoperiod sensitive and short day result in reduced vegetative phase and onset of flowering. Moist and humid conditions are conducive for vegetative growth and drier conditions at time of flowering and pod development stage is favorable for successful crop. Rains at the time of flowering results in poor pollination and pod development and also favors development of pod borers The temperature range of 18-27$^0$C is desirable. There are varieties which can tolerate temperatures below 10$^0$C and above 35$^0$C. It can be cultivated on variety of soils from sand to heavy clay loam; however well drained, medium heavy loams are the best suited. They are tolerant to water logging, frost and salinity.

**Anthesis and Pollination:** The flower structure of pigeon pea was initially adopted to self pollination, which changed over time to partial out breeding. In 90% flowers, pollination takes place before the opening of flower. The duration of flower opening varies according to climate and environment. Maximum anthesis takes place between 10.30 AM to 12.30 PM. Flowers that open in the evening usually remain open throughout the night and are closed before noon on the following day. The receptivity of stigmas starts 68 hours before anthesis and continues for 20 hours after anthesis**.**

**Package of practices for raising the crop**

**Season:** Normally the season in which the seed multiplication is to be taken up should result in high quality seeds

**Land requirement:** For nucleus and breeder seed production preferably one single field should be earmarked for a particular variety and should not have been used for other variety in the previous year.

| | | |
|---|---|---|
| Northern India | : | 2$^{nd}$ fortnight of May to 1$^{st}$ week of June |
| Eastern India | | |
| Early | : | 1$^{st}$ fortnight of June |
| Late | : | 1$^{st}$ fortnight of July |
| Pre-rabi | : | 1$^{st}$ fortnight of September |
| Central India | | |
| Rainfed | : | 1$^{st}$ fortnight of July (onset of monsoon) |
| Irrigated | : | 2$^{nd}$ fortnight of June |
| Southern India | : | Onset of monsoon (2$^{nd}$ fortnight of June) |

**Seed Rate**

| | | |
|---|---|---|
| Early | : | 18-20 kg/ha |
| Late | : | 12-15 kg/ha |
| Pre-rabi | : | 25-30 kg/ha |

**Spacing**

| | | |
|---|---|---|
| R-R | : | 60-75 cm |
| P-P | : | 10-15 cm |

**Fertilizers:** 20 kg N, 40-60 kg $P_2O_5$, 20 kg $K_2O$, 20 kg S/ha

**Irrigation:** Avoid water stress at the time of flowering and pod development stages

**Weed management:** Two hand weeding at 25 and 45 days after sowing (DAS) or application of STOMP (Pendimethalin) @ 3.0 l /ha at pre-emergence stage, followed by one hand weeding at 45-60 days after sowing.

**Plant protection:** Pod borer (*Helicoverpa armigera*), pod fly (*Melanagromyza obtusa*), wilt (*Fusarium udum*), sterility mosaic virus and blight (*Phytophthora drechsleri* var *cajan*) are major insect pests and diseases affecting the crop in different growing areas

**Disease management:** Field sanitation, destruction of all stubbles and plant remains, removal of off-season volunteer plants from vicinity. Seed treatment with Carbendazim + Thiram (1+2 g/kg seed) for wilt and other soil borne diseases. Planting on ridges in high rainfall area to reduce damage by Phytophthora blight

**Insect pest management:** North India:First spray of Dimethoate (0.03 %), followed by second spray of NPV (350 LE/ha) for control of pod fly and borer
Central / South India: First spray of NPV (350 LE/ha), followed by second spray of Dimethoate (0.03%) against pod borer and pod fly

**Time of spray:** At economic threshold level. ETL for pod fly is egg lying on 5% pod borer is 1-2 larva/plant. Spray interval should be 15 days or as per infestation.
For control of pod borer, application of chemical sprays (endosulfan @ 350 g a.i. ml $ha^{-1}$, Indoxacarb @ 70 ml a.i. $ha^{-1}$ (TRACER-200 ml) or Spinosad @ 45 ml a.i. $ha^{-1}$ (AVAUNT @ 400-500ml) can be applied as and when needed.

**Table 3: Field and seed standards of pulse crops**

| Standard/parameter | | Chickpea | | Urd | | Mung | | Lentil | | Peas | | Pigeonpea | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | C | F | C | F | C | F | C | F | C | F | C |
| **1. Field Standards** | | | | | | | | | | | | | |
| a) | Isolation (m) | 10 | 5 | 10 | 5 | 10 | 5 | 10 | 5 | 10 | 5 | 200 | 100 |
| b) | Off types (% )* | 0.10 | 0.20 | 0.10 | 0.20 | 0.10 | 0.20 | 0.10 | 0.20 | 0.10 | 0.20 | 0.10 | 0.20 |
| c) | Plants effected by seed borne diseases** ( % ) | - | - | - | - | 0.10 | 0.20 | - | - | - | . | - | - |
| **2. Seed standards** | | | | | | | | | | | | | |
| a) | Pure seed (min.) % | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 |
| b) | Inert matter (max.) % | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| c) | Other crop seeds/Kg (max.) | None | None | 5 | 10 | 5 | 10 | 5 | 10 | None | 5 | 5 | 10 |
| d) | ODV (max.) /Kg | 5 | 10 | 10 | 20 | 10 | 20 | 10 | 20 | 5 | 10 | 10 | 20 |
| e) | Weed seeds (max.) /Kg | None | None | 5 | 10 | 5 | 10 | 10 | 20 | None | None | 5 | 10 |
| f) | Germination (min.) % | 85 | 85 | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| g) | Moisture (max.) in % | 9.0 | 9.0 | 9.0 . | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 |
| h) | Vapour proof in % containers (max) | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

*Maximum permitted at the final inspection

****Seed Borne diseases**

| | |
|---|---|
| **Moong** | 1. Halo blight (*Pseudomonas phasiolicola* (Burk) Dows.) |
| **Cow pea** | 1. Ashy stem blight (*Macrophomina phaseoli* (Maub.) Ashby)<br>2. Anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav.)<br>3. Ascochyta blight (*Ascochyta* spp.) (for hill areas only)<br>4. Cowpea mosaic |
| **Indian bean** | 1. Bacterial blight (*Xanthomonas* spp.)<br>2. Anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magn Br. & Cav.)<br>3. Ascochyta blight (*Ascochyta* spp.) (for hill areas only) |
| **Rajmash** | 1. Bacterial blight (*Xanthomonas* spp.)<br>2. Anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav.)<br>3. Ascochyta blight (*Ascochytaphaseolorum* (Sacc.) Michelia) (for hill areas only)<br>4. Bean mosaic (*Macrosiphum pisi* Kalt.) |

# PRINCIPLES AND PRACTICES IN VEGETABLE SEED PRODUCTION

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## Introduction

Vegetables occupy an important place in diversification of agriculture and have played a pivotal role in nutritional security. With the changing paradigms of food and nutritional securities, the consumption of vegetables have attained tremendous importance. To meet the ever increasing demand of burgeoning Indian population, production and productivity of vegetables has to be increased manifolds. Due to increasing pressure on land through urbanization and industrialization, it is not feasible to increase the area under vegetables commensurate to our requirements hence the preciousness of high quality vegetable seeds becomes much more significant than it has ever been to increase the yield per unit area. Although use of quality seeds of improved varieties of different vegetable crops has witnessed tremendous growth in vegetable production and productivity, however, the availability of quality seeds in time and at affordable price is still a matter of great concern. Hence, it is imperative to enhance our vegetable seed production. For the pursuit, involvement for seed production at all the levels should be encouraged and for this well established principles and practices for vegetable seed production are the need of the hour.

The availability of quality seed is of utmost importance for increasing the vegetable production. Vegetable growers recognize quality seed of improved varieties as the most strategic resource for higher and better vegetable yields. Seed quality is the possession of seed with required genetic and physical purity that is accompanied with good germinability, health status and minimal moisture content. The growth of plant and the quality of seed production are strongly influenced not only by agronomic or genetic factors but also by the environmental condition, in which production is undertaken. Emphasis should always be laid on those factors which contribute to and affect seed quality like selection of crop and variety, seed source, roguing, harvesting and postharvest operations etc. The package of seed production technology vary from location to location and from crop to crop. But a broad general recommendation can be adopted which could be suitably modified on the basis of individual vegetables and the growing area.

## General principles of vegetable seed production

Production of genetically pure and otherwise good quality pedigree vegetable seed is an exacting task requiring high technical skills and comparatively heavy financial investment. During seed production strict attention must be given to the maintenance of genetic purity and other qualities of seeds in order to exploit the full dividends sought to be obtained by introduction of new superior crop

plant varieties. In other words, seed production must be carried out under standardized and well-organized condition. The major aspects to be taken care of during the vegetable seed production are the genetic and agronomic factors, if followed in a right way will end up in successful vegetable seed production.

**Genetic Principles**

These principles highly depend on the genetic characters of seed which can modify its performance in production programme. In seed production genetic characters are evaluated through genetic purity. The important factors causing the deterioration of variety are natural outcrossing, mechanical mixtures and selective influence of disease and thereby reduces chances of obtaining true to type seeds. Other factors which have minor influence include developmental variations, mutations, minor genetic variations and techniques of plant breeder.

**Management of genetic purity in vegetable seed production**

***Control of seed source****:* Appropriate class of seed purchased from approved source should be used for raising the seed crop. Breeder seed used for raising foundation seed plot and foundation seed used for raising certified seed plot. Seed must be purchased from approved sources like ICAR institutes, State Agril University, Agril. Research Station or State Seed Corporation. (SSC).

***Land requirement:*** There should not be same crop in the previous season to save genetic contamination from volunteer plants. They are unwanted plants of the same crop growing in the seed field from the seeds that remain in the field from previous year crop. These may act as a source of natural outcrossing there by reducing the genetic purity.

***Isolation distance:*** It helps to avoid natural crossing with undesirable plants, as well as to avoid mechanical mixture during sowing and harvesting. The seed crop must be isolated from other nearby fields of the same crop and other contaminating crops as per requirements of certification standards. The isolation of a seed crop can be provided by time, space and barrier between seed fields and contaminating fields. Isolation distances followed in some important vegetable crops are mentioned below (Table 1).

**Table 1:** Recommended isolation distance for foundation & certified seed production of different vegetables.

| **Vegetable Crops** | **Minimum isolation distance (meters)** | |
|---|---|---|
| | **Foundation seed** | **Certified seed** |
| Cowpea | 10 | 5 |
| Garden pea | 10 | 5 |
| Chilli & Capsicum | 400 | 200 |
| Cauliflower, Cabbage and Knol khol | 1600 | 1000 |

| | | |
|---|---|---|
| Lettuce | 50 | 25 |
| Carrot | 1000 | 800 |
| Onion | 1000 | 500 |
| Radish and Turnip | 1600 | 1000 |
| Okra | 400 | 200 |
| Tomato | 50 | 25 |
| Brinjal | 200 | 100 |
| Cucurbits | 1000 | 500 |
| Spinach beet (Palak) | 1600 | 1000 |

***Field inspection***: Field inspection and approval of growing crops at critical stages for verification of genetic purity, detection of mixtures, volunteer plants, weeds, freedom from noxious weeds and seed borne diseases. Table 2 shows the maximum permissible limits and roguing stages in vegetable crops.

**Table 2:** Important roguing stages and permitted off types for production of different vegetable seeds.

| **Vegetable crop** | **Minimum number of field inspections and roguing stages** | **Off type (%) max. permitted** | |
|---|---|---|---|
| | | **F** | **C** |
| Cow pea | 2: from flowering to fruiting | 0.10* | 0.20* |
| Garden pea | 3: 1st before flowering 2nd at flowering 3rd edible pod stage | 0.10* | 0.50* |
| Chilli and capsicum | 3: 1st before flowering, 2nd at flowering and 3rd at the mature fruit stage | 0.10* | 0.20* |
| Cauliflower, cabbage and knol- khol | 4: 1st before the marketable stage, 2nd start of curd head formation, 3rdwhen most plants have formed curd and 4th at flowering stage | 0.10* | 0.20* |
| Lettuce | 3: 1st before full grown stages in nonheading types, 2nd full grown stage in non-heading type and 3rdat flowering | 0.10* | 0.20* |
| Carrot | 4: 1st 20-30 days after sowing, 2nd at lifting and replanting, 3rd at flowering and 4th at maturity to verify the true nature of umbels | 0.10* | 0.20* |
| Onion | 4: (When seed crop is raised by the transplanting method) 1stearly (20-30 days after sowing), 2nd when bulbs are lifted, 3rd when bulbs are replanted and 4th at flowering | 0.10* | 0.20* |
| | 3: (When seed crop is raised by seed to seed method) 1st 20-30 days after sowing, second when bulbs are formed and 3rd at flowering | | |

| | | | |
|---|---|---|---|
| Radish and turnip | 3: 1st20-30 days after sowing, 2ndwhen lifted and replanted and 3rdat flowering | 0.10** | 0.20** |
| Okra | 3:1stbefore flowering, second at full flowering and fruiting and 3rd at mature fruit stage. | 0.10** | 0.20** |
| Tomato | 3: 1st before flowering, 2nd during flowering and the immature fruit stage and third at mature fruit stage | 0.10** | 0.20** |
| Brinjal | 3: 1st before flowering, 2nd during flowering and the immature fruit stage and third at mature fruit stage | 0.10** | 0.20** |
| Cucurbits | 3: 1st before flowering, 2nd during flowering and the immature fruit stage and 3rdduring mature fruit stage | 0.10** | 0.20** |

(*Maximum permitted at the final inspection; ** Maximum permitted at and after flowering, F-foundation seed, C- certified seed)

***Roguing***: Adequate and timely roguing is extremely important in seed production. As mentioned earlier the rogue which differ from normal plant population in being weak or sickly or bolters or dissimilar may cause quick deterioration in seed stocks by cross pollination, transmission of diseases etc. they should therefore be removed at earliest possible date before flowering. It is wise to remove the whole plant and not just the flower head. The number of roguing will vary with the crop, cleanness of planting seed and stage of the multiplication of the seed crop. Roguing in most of the field crops may be done at any of the following stages as per needs of the seed crop.

a. Vegetative/pre-flowering stage
b. Flowering stage
c. Maturity stage

**Agronomic principles**

Standardized seed production, besides genetic principles involves the application of the following agronomic principles to preserve good seed quality and abundant seed yields.

**Selection of suitable agro-climate/ region:**

Vegetable (variety) grown for seed production in an area must be adapted to the photoperiod and temperature conditions prevailing in that area. Regions of moderate rainfall and humidity are much more suited to seed production than regions of high rainfall and humidity. It may also result in delayed maturity and pregermiantion of seed in many standing crops. Most crops require a dry sunny period and moderate temperatures for flowering and pollination. Excessive dew, rain cause hindrance in normal pollination, too high temperature causes

desiccation of pollen and hot dry weather conditions prevail during flowering results in poor seed set.

**Selection of field:**

The soil texture, structure and pH should be considered while selecting field for vegetable seed production and soil should be moderately fertile. The seed plot should be free from soil-borne diseases and insect pests, volunteer plants, weed plants and other crop plants. It must have an assured irrigation source. Location should have transportation facility (Rail, road etc.). There should be no forest reserve near to seed plot.

**Seed rate and spacing:**

The requirement of seed rate in the vegetable seed production is always less than commercial crop production. Generally large sized seeds are grown in situ. Seed rate is high in direct sown crops compared to transplanted crop. Seed rate depends on season of planting, duration and spread. Spacing for each crop/variety is fixed based on the spread of plants and by considering easiness for cultural operations like weeding, fertilizer application and harvesting etc. Table 3 contains information on seed rate and spacing in different vegetable crops.

**Seed treatment:**

The seed may require seed treatment before planting, if they are not already appropriately treated. This could be easily done with help of a revolving drum at the farm. While treating the seeds care must be taken to apply the combination of chemicals, bioagents in a correct order viz., fungicide-insecticide-rhizobium. Depending upon the requirement one or more of the following seed treatments may be given:

a. Chemical seed treatment: fungicide/ insecticide
b. Bioagents: *Trichoderma, Pseudomonas*,etc
c. Bioinoculant: *Rhizobium*, PSB, *Azatobacter*, VAM etc.

**Method of sowing:**

***I. Directed seeding****:*

Large seeded vegetables like squash and other cucurbits including melons, root vegetables such as carrot and radish, many leafy vegetables, okra and bitter gourd are sown by direct seeding. There are different methods of sowing which needs to be selected based on crop type, irrigation availability. In vegetable seed production, broadcasting should be avoided as it poses difficulty in organizing the cultural operations.

**a. Line sowing:** Line sowing is essential and has following advantages

**1)** Seeds are placed at proper & uniform depths

**2)** Along the rows, interculturing can be done

**3)** Uniform row to row spacing is maintained
**4)** Seed requirement is less than 'broad casting'
**5)** Sowing is done at proper moisture level.

**b. Ridge planting:** In ridge plant, crops are planted into ridges formed during cultivation of the previous crop. Ridge planting reduces erosion by leaving the soil covered with residue until planting. Gently sloped fields, especially those with poorly drained soils, are well suited to ridge systems. This systems complement furrow irrigation. Eg: root crops- radish, carrot, beetroot, turnip, potato

**c. Raised bed planting:** Vegetable crops can be grown on raised-up beds. They are especially advantageous for clayey soils under high rainfall or wherever else drainage is likely to be poor. The vegetables grown on raised bed are solanaceous vegetables, cole crops, onion and garlic. Raised beds may have several advantages viz.,

• Much better drainage compared with flat or sunken beds.
• They provide a double layer of topsoil, because they're made by dragging in topsoil from the surrounding alleyways. (Because of this, they're also likely to be looser than flat or sunken beds)
• In temperate regions, raised beds warm up more quickly in the spring, which may benefit cold-sensitive crops and even permit earlier planting.
• Plants on raised beds are easier to reach when doing hand operations such as weeding and thinning.

**d. Flatbed sowing:** Flat beds are used where water availability is adequate and there are no drainage problems. In some areas, crops like leafy vegetables, garden pea, beans and potatoes are started out on a flatbed; as the season progresses, soil is thrown into the crop row to mound up the plants; this is called "hilling-up" and is done to control in-row weeds, provide support, and improve drainage. (Potatoes are also hilled up to keep the developing tubers covered with soil.) Hilling-up only works with plants that have enough stem height and leaf clearance to tolerate partial burial. Eg: leafy vegetables, garden pea, beans.

**e. Hill channel sowing**: The 'channel and hill' system of cultivation is most useful and scientific for higher yield. After preparing the field, 45cm wide and 25-30cm deep channels are made preferably from east to west 3.0-4.5m apart and slope on northern side of used for sowing. Eg- cucurbits, dolichus bean etc.

**II. Transplanting**: Eg- small seeded vegetables like tomato, chilli, capsicum and brinjal are transplanted by raising nursery in open as well as protected conditions.

**Staking and trailing:**

Staking is practiced in hybrid seed production of tomato, chilli, capsicum, brinjal for better management of crop and pollination. While trailing is helpful in cucurbits and dolichus bean (pole type) and can be trained with the help of different type of structures.

**Weed management:**

***Using mulch:*** The use of plastic mulching is very popular in many vegetable-growing areas. A non-transparent plastic is used to impede the transmission of photosynthetic radiation through the plastic to the weeds so that the development of weeds is then arrested. It should be noted that one application will not be sufficient against perennials.

***Use of weedicide*:** There are two types of weedicides which are applied as a pre-emergence/ post emergence. These are applied for best management of weeds in onion, garlic, leek, cole crops and pepper etc.

a. **Pre-emergence**: Use of Alachlor @ 1.0 kg $ha^{-1}$, Pendimethalin @ 1.8 kg $ha^{-1}$, Metribuzin @ 1.0 kg $ha^{-1}$ and Oxidiazon @0.75 kg $ha^{-1}$.
b. **Post-emergence**: Paraquat and Diquat @ 0.36-1.0 kg $ha^{-1}$, Metribuzin @ 0.35-1.0 kg $ha^{-1}$.

**Irrigation**:

There are different methods of irrigation like drip, flooding and sprinkler system but mostly followed and suitable is the drip irrigation. Flooding is followed in leafy and solanaceous vegetables. Foliar /sprinkler irrigation should be avoided in vegetable seed production as it leads to flower drop. During flowering period in vegetables like beans, tomatoes, cucurbits etc. it's critical to supply irrigation otherwise leads to defoliation. Another critical period is the development of fruit or seed. For heading crops such as lettuce and cabbage, the most critical period at heading stage. An adequate amount of water is essential for root crops once the root starts enlarging. To protect crop from frost, provide light irrigation.

**Nutrient management:**

In the nutrition of seed crops nitrogen, phosphorous potassium and several other elements play an important role for proper development of plants and seeds. It is therefore advisable to know and identify the nutritional requirements of seed crops and apply adequate fertilizers. Adequate fertilization results in maximum yields, god seed quality and better expression of plant type which facilitate roguing and thereby helps in maintaining higher genetic purity as well (Table 3). During sowing or preparation of land half of N and full amount of P and K should be applied. Remaining half N should be applied at 35/ 55 days after planting. Micronutrients are essential for plant growth, but plants require relatively small amounts of them. They include boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn). Deficiency of these will lead to problems like pollen sterility, flower drop and stunted growth. During frost and fog conditions potassium or sulphur spray 1-2% would increase the stress tolerance. Table 3 below shows the requirement of NPK in different crops along with micronutrients wherever necessary to be applied.

**Table 3:** Seed Rate, Planting Distance and fertilizer application in different vegetable crops.

| **Crop** | **Seed rate/ha** | | **Spacing (Cm)** | **Fertilizer (kg/ha) ($N:P_2O_5:K_2O$** | **Others** |
|---|---|---|---|---|---|
| Tomato | 400g | | 60x30(Det.) 100x60 (Indet.) | 120:60:60 | 20kg/ha borax & 0.5% Zinc/ha |
| Brinjal | 500g | | 100x90 | 120:60:60 | |
| Chilli | 650g | | 45x30 | 120:80:60 | |
| Capsicum | 700g | | 60 x30 | 120:80:80 | 20kg/ha borax & calcium |
| Vegetable Peas | 100-160 Kg | | 23x5-7 | 60:60:60 | |
| French Bean | Bush | 50kg | 45x15 | 120:60:60 | |
| | Pole | 40kg | 60x30 | | |
| Cowpea | Bush | 20kg | 45x15 | 40:60:60 | |
| | Pole | 15kg | 60x30 | | |
| Okra | Summer | 18kg | 60x15 | 120:60:60 | |
| | Rainy | 10kg | 60x30 | | |
| Cauliflower | Early | 600g | 40x30 | 120:60:60 | 25kg/ha borax &1.5kg/ha A. molybdate |
| | Late | 400g | 60x45 | | |
| Cabbage | Early | 600g | 40x30 | 150:60:60 | |
| | Late | 400g | 60x45 | | |
| Radish | Sowing | 400g | 45x8 | 100:50:50 | 10kg boron/ha |
| | | | 75x60 | 100:80:60 | |
| Carrot | Steckling | 500g | 50x10 | 80:60:80 | |
| | | | 75x60 | 60:60:80 | |
| Onion | 650g | | 45x30 (Bulb spacing) | 100:60:60 | |

**Crop protection:**

Successful disease and insect control is another important factor in raising healthy seed crops. Apart from reduction in yield, the quality of seeds from disease and insect damaged plants is invariably poor. Poor disease and insect control affects seed quality by perpetuating in the seed coat or soil which again hits back in next season. Following principles may be adhered for an effective management of diseases and pests in seed crops:

a. Plant only treated seed.

b. Prepare and adopt appropriate schedule of spraying for effective disease and insect control.
c. Roguing of diseased plants and ear heads from time to time also helps in checking the further spread of diseases.
Use of imidacloprid 17.8%SL (25g.a.i/ha) and thiomethaxam 25%WG (15g.a.i/ha) are used for control of jassids, thrips and aphid in chilli, okra, tomato and brinjal. Spinosad 45%SC used against diamond back moth in cauliflower/cauliflower and fruit borer in chilli. Need based soil drenching with captan 75 WP @ 0.25 % or captan 75 WS @ 0.2-0.3 % or mancozeb 75 WP @ 0.3 % or metalaxyl + mancozeb @ 0.3 % to manage damping off / rots. Spray mancozeb 75 WP or zineb 75 WP @ 1.5-2 kg / ha in 750-1000 lit water to manage leaf spot and blights.

**Harvesting and threshing:**
Proper post-harvest processing is critical to maximize yield, longevity, vigor, and overall quality of the seed crop. At maturity, seed must be harvested, threshed, cleaned, and fully dried before storage. Vegetable crops which have determinate growth habit will be harvested at once, eg: garden pea, spinach beet, methi, broad bean, french bean and cole crops. Indeterminate types will be harvested multiple times periodically. Eg: solanaceous vegetables (tomato, chilli and capsicum), dolichus bean, cow pea (indet.).Vegetable seed crop will be harvested at harvestable maturity (HM). After harvesting 1-2 days of shade ventilation should be given to reduce moisture and after this seeds are subjected to artificial drying. Initial drying will be started from lower temperature of 28 $^{o}$C and it may reach upto 40 ± 2 $^{o}$C.

**Seed extraction:**
Various types of threshing machines with adjustable cylinder speeds are available for extraction of vegetable seeds. The cylinder clearance, concave mesh size, airflow rate and screen size greatly influence the efficiency of these machines. Every care must be taken to avoid damage to the seed during mechanical threshing, by properly adjusting the speed of the beaters, the width of the gap between the beaters and the concave, the airflow and the sieve sizes.

***1. Dry seed separation***

Common method mostly performed by women labour. Relatively cheap, easy and make use of surplus local labour. Usually adopted for threshing high value vegetable seeds. Hand threshing may be done in the following ways.
**a. Rubbing:** Rubbing seeds materials with a pressure in an open-ended trough line with ribbed rubber (bamboo contained). This method is quite suitable for pod materials such as brassicas and radish.
**b. Beating:** The seed materials is beaten with the help of wooden pliable sticks repeatedly with a tolerable force as the seeds are separated but not broken.

**c. Flailing:** Specially designed instruments are used for separating the seeds from the plants.

**d. Rolling:** Seed materials is rolled on threshing floor or tarpaulin repeatedly and seeds are easily separated.

**2. *Wet seed separation***

**i. Manual Method**

(a) Maceration e.g., watermelon,
(b) Crushing e.g., brinjal,
(c) Scraping e.g., cucumber
(d) Separated e.g., muskmelon,
(e) Scooping e.g., pumpkins and
(f) Extraction e.g., squashes.

**ii. Dry Extraction:** Dry extraction is done either manually or mechanically. Manual extraction is by beating with pliable bamboo stick or by beating against a hard surface. Threshers (LCT) are used for mechanical extraction. In this method care should be taken to avoid mechanical injury.

**iii. Wet Extraction:** It is normally practiced in fleshy fruits of vegetables like tomato, brinjal, bittergourd, snakegourd and ashgourd. Among these, extraction is easier in brinjal and ashgourd as the fleshy pulp's interference is less. Seeds are separated with pulp and are washed with adequate water and for removing the sliminess; seeds are washed with 0.1% HCI for 2-3 minutes.

**iv. Fermentation Method:** Fruits with pulp and seed are squeezed and kept as such for 24-48 hours. The seeds will settle down. Decayed pulp and immature seed will float. The settled seeds are washed with more of water. The seeds are shade dried and then sun dried before using. Care should be taken to avoid germination of seed during fermentation. The seeds will be dull in color.

**v. Chemical method:**

**a. Alkali method**

This method is relatively safe and can be used for small quantities of seed in cooler temperate areas where the fermentation method is not used. The pulp containing the extracted tomato seed is mixed with an equal volume of a ten per cent solution of sodium carbonate (washing soda). The mixture is left for up to 48 hours at room temperature and after washed out in a sieve and subsequently dried. This method is not suitable for commercial seed production as sodium carbonate tends to darken the testa of the seed.

**b. Acid method**

Acid method is often favored by large commercial seed producers as it produces a very bright clean seed. Addition of 30ml of hydrochloric acid per litre of seed and pulp mixture, stirred properly and left for half an hour then the seeds are washed thoroughly with water, sieved and dried.

By following the principles of seed production we can assure that the seeds which are supplied to the farmers are of high quality and perform as intended.

# SEED PRODUCTION OF VEGETABLES AND ORNAMENTAL CROPS UNDER PROTECTED CONDITIONS

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Most of the vegetable and annual flower crops are grown by seeds and it is now well established fact that the seed is the most vital input for commercial crop production. Earlier the cost of seed was constituting only a fraction of the total cost of inputs involved in raising the crop, but now a days seed is contributing a good amount in the total cost of cultivation. The basic reason behind this fact is that the use of quality seeds and hybrids has increased sharply during last decade. The reason of high cost of hybrid and quality seed is basically the poor seed yield when they are produced in open field. Vegetable and ornamental seed crops faces tremendous challenges in the production of disease free, healthy and genetically pure seed for commercial cultivation. Conventional practices of seed production in open fields' faces common problems like lack of sufficient isolation, insects, diseases and a virus free environment. Compared to conventional practices, protected cultivation can deliver higher seed yield with better quality. Insects and viruses are the most devastating problems for quality seed production in most of the vegetable and ornamental crops in open fields, and if the insect vectors are checked by using protected structures this problem could be solved with less application of pesticides. An innovation applicable in a very practical manner is always likely to get popularity to the assumed potential resulting in significant profit to the stakeholders. Seed production under protected conditions is a best example of it. A decade ago, research on seed production under protected conditions was initiated in India and tremendous work has been carried out to standardize the technology, i.e. for quality seed production realizing the various biotic and abiotic stress problems encountered in open field seed production programmes.

The present article provides information on quality seed production of vegetables and ornamental crops under various protected and open field environment. This concept has shown significant results not only in quality seed production but also in increasing the per unit seed yield in different vegetables and ornamental crops. The protected cultivation models has proved to be highly economical and profitable with limited resources and man power to provide best quality seeds of numerous vegetables and ornamental crops cultivated across the globe.

## What is protected cultivation?

When we raise a crop and to protect the crop from major biotic and abiotic stresses is termed as as protected cultivation. There are several other

advantages of protected cultivation viz. high productivity,high quality and long duration availability, off season cultivaton,judicious use of natural resources,more employment generation,minimal use of chemicals etc.

Protected cultivation is becoming more important for cultivation of seed crops compared to commercial crops due to continuous climate change. Therefore, protected cultivation of vegetable and ornamental seed crops provides the best opportunity to grow virus free seed crops and to produce much better quality seed compared to open fields. Similarly, when the seeds crops are almost ready for harvesting and if there are seasonal rains, they can completely spoil the seed quality. Under such situations protected conditions like greenhouse, walk in tunnels provides the best opportunity to protect the seed crops from such calamities. Therefore, different kind of protected structures can be used to fulfill different objectives in various regions.

Types of protected structures suitable for quality seed production in vegetable and Ornamental crops :

1. Climate controlled green house
2. Semi climate controlled green house
3. Naturally ventilated greenhouse
4. Insect proof net house
5. Walk-in-tunnel
6. Plastic low tunnels
7. Rain shelters
8. Cage Houses

High cost of seed is mainly due to poor seed yield in different vegetables in open field seed production programs. Suitable areas for quality seed production of important vegetables are also limited and in these areas too climate change and biotic and abiotic stresses have become a major problem in the way of quality seed production in open field conditions. The threat of climate change and its impact on agriculture is increasing and becoming a common phenomenon day by day and under such situation quality seed production in vegetable and ornamental crops is going to be a major challenge. Therefore, it is of utmost importance to develop advanced technologies or methods for quality seed production, increasing the seed quality and seed yield in major vegetables and ornamentals in the event of continuously changing climatic conditions and increasing threat of new viruses, insects/ pests and diseases in the country. Looking at these problem, recently there has been a technical shift towards the production of high quality hybrid seeds of important high value vegetables and flowers under protected conditions. With time it has also been proved that protected cultivation is a better technology to enhance land productivity and quality of the produce by providing a logical and technical solution to manage the major biotic and abiotic stresses encountered in open field cultivation of vegetable crops. The effectiveness of the technology has been observed world over for commercial cultivation of various horticultural crops. Hence, the area

under protected cultivation in the last decade in various parts of the world has increased exponentially particularly in countries like China due to adoption of various protected cultivation technologies in different forms like mulching, use of temporary plastic walls in open fields, low tunnels, walk-in-tunnels, insect proof net houses, shade net houses and greenhouses. But, the selection criteria of protected structures to be used for vegetable and ornamental crop seed production certainly depends upon few important factors, viz. climatic conditions of the area selected for seed to be produced, vegetable /ornamental crop, type of seed crop, season of seed production and targeted quantity of seed production. Although, very limited work has been done throughout the world for comparing the seed yield and quality of the seeds of various vegetables and ornamentals grown in open field vs under protected conditions. But whatever work has been done on this aspect clearly reflects that there is a definite gap in the seed yield and seed quality grown under protected conditions in comparison to open fields.

**Healthy nursery raising of vegetables for quality seed production:**

Healthy and virus free seedlings of the seed crop in case of OP varieties and healthy seedlings of the parental lines in case of hybrid seed production is utmost important for quality seed production in vegetable and ornamental crops. Plug tray nursery raising technology has been standardized for different groups of crops under greenhouse conditions. Nursery is raised using soil less medium under protected conditions. The common medium used is mixture of cocopeat, perlite and vermiculite in 3:1:1 ratio. Using the mixture seedlings of different vegetable and ornamental crops are raised and then crop is grown in different protected structures. Mostly cucurbits, Solanaceous, cruciferous vegetables and Bigonia,Primula,Geranium,Pansy,Marigold and ornamental kale seed is produced under protected structures.

**Fig.1** Multiplication of parent line in Insect proof net

**Fig. 2** Cherry Tomato in Polyhouse **Fig. 3** Insect proof net house

**Fig. 4**. Seed production of tomato under Naturally ventilated polyhouse

**The major advantages of seed production under protected conditions are:**

(i) High seed yield generally 2-4 times more compared to open field in major vegetables.

(ii) Quality of seed is always high compared to open field produced seed.

(iii) Requirement of large isolation distance in cross pollinated vegetables can be minimized under protected conditions.

(iv) Problem of synchronization of flowering in parental lines can be minimized.

(v) Maximum plant population can be maintained along with appropriate ratio of male and female parents for higher hybrid seed yield.

(vi) Seed production under adverse climatic conditions is possible where it is not possible in open field conditions.

(vii) Duration of seed crop is more under protected conditions when compared with open field seed crops.
(viii) Field standards could be enforced well under protected conditions and healthy virus free seed crop can be grown, which is very difficult under open field conditions.
(ix) Training, pruning and hand pollination practices are very easily manageable under protected conditions compared with to field seed crop.
(x) More number of seed crops could be grown under protected conditions.
(xi) By using soil less media, seed crops can also be grown even under saline and acidic soil conditions.
(xii) Protected conditions provide the best opportunity for organic seed production in vegetables.
(xiii) Judicious use of natural resources like soil and water is possible for seed production under protected conditions.
(xiv) Virus free healthy seedling production of the parental lines is possible under protected conditions.
(xv) Grafting technology in seedling could be easily applied to overcome soil borne problems in solanaceous and cucurbitaceous vegetables.
(xvi) Quality seed production of vegetable and ornamental crops could be under taken by agri-entrepreneurs in various regions of the country and this in turn could generate lot of employment opportunities in rural areas.
(xvii) Seed production cost of vegetables /ornamentals could be reduced by growing seed crop under protected conditions.
(xviii) In hybrid seed production, emasculation of female parents is not required as there are no insect pollinators inside the protected structures.
(xix) Complete protection of the crop from rodents and birds compared to open field seed crops.
(xx) Seed crops will not be damaged by un-seasonal rains at the time of their maturity unlike open field seed crops.
(xxi) Off type plants, objectionable weeds or plants affected by designated diseases cannot pose problems in the seed crops raised under protected conditions.
(xxii) Seed viability and seed vigour could be extended through better nutrient management in seed crops under protected conditions.

The seed yield and quality is always better when seed production is undertaken in protected conditions. Semi climate controlled greenhouses were found to be ideal for indeterminate type hybrids/varieties of standard tomato, cherry tomato, sweet pepper and parthenocarpic cucumber. Seed yield of such crops could be increased 2-3 times compared to the open field. Beside the quality of seed is also superior. Similarly, zero energy naturally ventilated greenhouses have also been found equally suitable for hybrid seed production of high value vegetable and ornamental crops. Here also the seed yield is also usually 2- 3

times more than open fields, but the cost of seed production could be only half (1/2) of the seed produced under semi-climate controlled greenhouse conditions.

Naturally ventilated green houses with good side and top ventilation could be used in northern and central plains of India, whereas insect proof net houses could be used for large scale seed production programs in low rainfall areas including arid and semi-arid regions of the country. Insect proof net houses were found to be most efficient among the low cost protected structures suitable for quality hybrid or seed production of OP and hybrid varieties of a large number of vegetables, viz. tomato, sweet pepper, chilli,okra, brinjal and important cucurbits and ornamental crops.

The major advantages of seed production under insect proof net houses are virus free seed crops, protection of seed crop against other major insects/pests like shoot and fruit borer in brinjal, fruit borer in okra and tomato, fruit fly in cucurbits, leaf minor in cucurbits and solanaceous vegetables and red pumpkin beetle in cucurbits. Not only this, insect proof net house also provides protection to the seed crop even under mild frost conditions. Insect proof net houses can be developed as temporary or permanent structures according to regional requirements. Low cost temporary protected structures like walk in tunnels and plastic low tunnels could be used for off season seed production or advancing the season of seed production, in northern plains of India during winter season.

This clearly reflects the advantages of seed production under protected conditions.

# PRINCIPLES AND PRACTICES OF SEED PRODUCTION IN FORAGE CROPS

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## Introduction:

Livestock is an important component of Indian agriculture contributing 4.4% of total GDP of the country, which amounts to 25% of Agriculture GDP (Economic survey, 2015-16). India, with 2.29% of the world land area, is maintaining about 10.71% of the world's livestock population. The number of milch animals have increased from 62 million in 2000 to 83.15 million in 2012. In 2014-15, 146.3 million tonnes of milk, which is 18.5% of total world milk, was produced with a year on year growth rate of 6.26% (Economic survey, 2015-16). However, as per an estimate India needs 400 million tonnes of milk by 2050. Even though we are first in milk production, our productivity (1538 kg/year) is far low than world average (2238 kg/year). One of the main reasons for this low productivity is lack of sufficient feed and fodder resources. At present the country is facing severe deficit in feed and fodder with 35.6% shortage in green fodder, 26% in dry-crop residues and 41% of concentrates. The stagnation in cultivated area under fodder crops from decades onwards is culprit for massive green fodder deficiency. The degradation of natural grazing lands because of urbanization, expansion of cultivable area, grazing pressure, industrialization etc. aggravated the situation further. To reduce the demand and supply gap, the production and productivity of fodder crops needs to be enhanced. The increase in cultivable area of fodder crops is difficult because of severe competition from food crops. Apart from vertical expansion, utilization of non-cultivable areas for pastures is one of the most viable options to balance the demand. India possess nearly 85 m ha of grasslands/ rangelands and forest wastelands, which are mostly in degraded state. Revitalizing these denuded lands is the most plausible means to improve the availability of green fodder. Grasslands also play pivotal role in the conservation of natural resources by preventing the denudation of degraded land mass and thus preventing soil erosion, enhancing bio-diversity and increasing carbon sequestration. The wider use of perennial range grasses selected for their special utility in the diverse land and climatic situations found in the arid and semi-arid tropics require seed or planting material of good quality and its continued availability to farmers through trade or farmer's own initiatives in multiplying material for his own use (Parihar, 2010). For wide spread regeneration of marginal and uncultivable wastelands, forest lands and grass/range lands, seed is the best propagating material. One of the reasons reported to stumble the green fodder production is non-availability of quality seed in sufficient quantities. As per an estimation, availability of quality seed is only 25-30% in cultivated fodder and <10% in range grasses and legumes in India (Anonymous 2011). Seed

production in fodder and grass species is comparatively difficult than regular food crops. Hence, there is a great need to understand the problems and innovate different mechanisms to mitigate them through research.

**Seed and fodder production potential of forages:**

Fodder is the food for domesticated animals including cut plants, hay, silage, straw, feed pellets, oil cakes, mixed rations, legumes etc. whereas forage is the plant material eaten by the grazing livestock. The common forage crops are grasses, legumes and fodder trees and shrubs. The seed yield is far low compared to green forage yield in both cultivated and range species (Table 1).

**Table 1:** Seed yield and green forage yield of important forage crops

| **Crops** | **Seed yield (kg/ha)** | **Green forage Yield (t/ha)** |
|---|---|---|
| **Cultivated Legumes** | | |
| *Berseem* | 400-700 | 60-100 |
| *Lucerne* | 150-260 | 60-110 |
| Cowpea | 700-1000 | 25-45 |
| Guar | 500-900 | 15-30 |
| **Cultivated Cereals** | | |
| Oat | 1200-2200 | 35-50 |
| Sorghum | 600-1700 | 55-70 |
| Bajra | 700-1000 | 20-35 |
| Maize | 1800-2200 | 30-55 |
| **Pasture grasses** | | |
| *Pennisetum purpureum* | 120-350 | 70-100 |
| *Panicum maximum* | 30-550 | 70-140 |
| *Setaria sphacelata* | 60-150 | 50-95 |
| *Penisetum pedicellatum* | 300-500 | 50-90 |
| *Bracharia mutica* | 35-60 | 100-190 |
| *Bracharia bryzantha* | 25-60 | 50-80 |
| *Cenchrus ciliaria* | 60-150 | 15-40 |
| *Cenchrus setigerus* | 50-90 | 10-30 |
| *Dichanthium annulatum* | 60-110 | 20-35 |
| **Pasture legumes** | | |
| *Lablab purpureus* | 300-400 | 15-25 |
| *Macroptelum atroperpureum* | 150-250 | 15-25 |
| *Stylosanthes hamata* | 500-1200 | 30-55 |
| *Clitoria ternatea* | 60-100 | 20-30 |

**Principles of seed production in forage crops:**

Forage crops comprises mainly two categories, i) cultivated and ii) range species. The seed production principles of cultivated forage crops is similar to

normal seed crops with minor deviations in the seed rate and spacing. However, the seed production of range species is altogether different and difficult due to intrinsic problems. There are mainly two systems of seed production for range species viz., opportunist system and specialist system (HSU, 1994).

**i) Opportunist system**

This is an informal system. In this system forage particularly range species seed harvesting will be taken up from existing grasslands/ rangelands. Seed will also be collected from road side pastures and plantations or other areas where the grasses/ legumes are abundant naturally. In this system no special plantation of forage crop will be there.

***Advantages:***

- This system yields seed at low cost
- No special care/ maintenance is required

***Disadvantages:***

- Inert matter content in the procured seed may be more particularly during sweeping/ raking the shattered seed from ground.
- Generally the seed will have low germination percentage
- Varied seed maturity occurs due to differences in plant and tiller maturity.

**ii) Specialist system**

In this system of seed production, forage crop is planted for seed production. This system requires technical guidance to grow the crop. For successful working of this system, it requires assured market and reasonable prices for the seed.

***Advantages:***

- High yields are possible in this system due to systematic crop management
- High quality seed are produced

***Disadvantages:***

- Availability of assured demand for forage seeds is the driving force for this system
- Farmers' preference for fodder crops is less due to less profitability, un-organised market, competition from food and commercial crops etc.
- Cost of production is more due high labour intensive methods.

In both the systems, following points are to be considered while taking up seed production.

- The seed production needs suitable climate such as congenial day length, which controls flowering in many forages and sunny weather, which is required for rapid growth, flower opening and increased bee activity.
- Suitability of forage: grasses can be produced in wide range of climates and soils than legumes. For tropical species, temperature is very

important factor. So based on the site of production suitable species should be identified.

- Crop establishment is the most difficult phase in range species. It requires good conditions for germination, emergence and growth. Pasture seed crops warrant more care during establishment with better land preparation, more precise sowing techniques, higher seeding rates and even additional operations (e.g. herbicides, irrigation) not normally used with pastures.
- Land clearing, seed bed preparation weed control in the initial stages, fertilizer application, time of sowing, choice of propagating material (rooted slips/ seeds), inoculation in case of legumes are some of the factors to be taken care.
- Sowing type: rows in case of tussock grasses and vigorous sprawling legumes, broadcasting in creeping legumes and stoloniferous grasses. The depth of sowing should not be more than 1 cm depth in small sized seeds.
- Under crop management, during defoliation of grasses bulk of the stubble should be removed at the beginning of the season so that the final clean cut can be less severe. In case of legumes defoliation should be done early enough to allow complete recovery of canopy and to avoid excessive vegetative growth. Nitrogen fertilization is necessary in grasses after defoliation.
- Seed harvesting: The choice of harvesting is complicated in range species due to their indeterminate growth and variation in the maturity levels within the inflorescence. Based on visual indicators and experience harvesting has to be done mostly either through hand picking or though cutting machines.

**Seed production package of some important fodder crops:**

| Crop | Suitable Season | Seed rate (kg/ ha) | Row Spacing (cm) | N:P:K (kg/ha) |
|---|---|---|---|---|
| Sorghum | Kharif | 10-15 | 30 | 90:30:0 |
| Maize | Kharif | 25-30 | 50 | 90:30:30 |
| Cowpea | Kharif | 20-30 | 50 | 60:40:30 |
| Guar | Kharif | 20-30 | 40 | 60:40:30 |
| Oat | Rabi | 50-60 | 25 | 90:30:0 |
| Berseem | Rabi | 20 | 35 | 20:80:30 |
| Lucerne | Rabi | 20 | 35 | 20:80:30 |
| Indian Clover | Rabi | 20 | 35 | 20:80:30 |
| *Cenchrus ciliaris* | Kharif/ perennial | 3-5 | 50 | 60:30:30 |
| *Cenchrus setigerus* | Kharif/ | 5-6 | 50 | 40:30:30 |

| | | | | |
|---|---|---|---|---|
| | perennial | | | |
| *Brachiaris brizantha* | Kharif/ perennial | 3-5 | 50 | 20:40:20 |
| *Pennisetum pedicel*latum | Kharif/ annual | 5-8 | 50 | 30:30:20 |
| *Clitoria ternatea* | Kharif/ perennial | 15-20 | 40 | 10:30:30 |
| *Panicum maximum* | Kharif/ perennial | 3-5 | 50 | 80:50:50 |
| *Siratro* | Kharif/ perennial | 3-5 | 40 | 10:30:30 |
| *Stylosanthes hamata* | Kharif/ perennial | 5-6 | 40 | 10:30:30 |
| *Chrysopogon fulvus* | Kharif/ perennial | 5-6 | 50 | 40:20:20 |
| *Dicanthium annulatum* | Kharif/ perennial | 4-6 | 50 | 20:20:20 |
| *Setaria* | Kharif/ perennial | 2-5 | 50 | 60:40:30 |

**Seed quality standards of important forage crops**

| Crop | Suitable Season | Isolation distance (m) | | Percent Off types | | Germination % | Purity % |
|---|---|---|---|---|---|---|---|
| | | FS | CS | FS | CS | | |
| Sorghum | Kharif | 200 | 100 | 0.1 | 0.2 | 75 | 97 |
| Maize | Kharif | 400 | 200 | 0.2 | 0.5 | 90 | 98 |
| Cowpea | Kharif | 10 | 5 | 0.1 | 0.2 | 75 | 98 |
| Guar | Kharif | 10 | 5 | 0.1 | 0.2 | 70 | 98 |
| Oat | Rabi | 3 | 3 | 0.05 | 0.2 | 85 | 98 |
| Berseem | Rabi | 400 | 100 | 0.2 | 1 | 80 | 98 |
| Lucerne | Rabi | 400 | 100 | 0.2 | 0.1 | 65 | 98 |
| Indian Clover | Rabi | 50 | 25 | 0.2 | 0.1 | 80 | 98 |
| *Cenchrus ciliaris* | Kharif/ perennial | 20 | 10 | 0.1 | 1 | 30 | 80 |
| *Cenchrus setigerus* | Kharif/ perennial | 20 | 10 | 0.1 | 1 | 30 | 80 |
| *Brachiaris brizantha* | Kharif/ perennial | - | - | - | - | - | - |
| *Pennisetum pedicel*latum | Kharif/ annual | 20 | 10 | 0.1 | 1 | 50 | 95 |
| *Clitoria ternatea* | Kharif/ perennial | - | - | - | - | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Panicum maximum* | Kharif/ perennial | 20 | 10 | 0.1 | 1 | 20 | 80 |
| *Siratro* | Kharif/ perennial | - | - | - | - | - | - |
| *Stylosanthes hamata* | Kharif/ perennial | 50 | 25 | 0.1 | 1 | 40 | 90 |
| *Chrysopogon fulvus* | Kharif/ perennial | 20 | 10 | 0.1 | 1 | 15 | 80 |
| *Dicanthium annulatum* | Kharif/ perennial | 20 | 10 | 0.2 | 1 | 40 | 90 |
| *Setaria* | Kharif/ perennial | 400 | 200 | 0.1 | 1 | 50 | 95 |

**Major problems in forage seed production:**

- Indeterminate growth: No synchrony in reproductive and vegetative growth resulting in lower partitioning of photosynthates to reproductive parts.
- Uneven maturity: non-uniform maturity with in inflorescence / panicle.
- Seed shattering: The matured seed shatters easily.
- Blank seed: No true seed formation only fluff due to poor ovule to seed ratio.
- Seed dormancy: Most of the range species have dormancy either physical or physiological in nature.
- Climatic factors: Seed production is highly influenced by the photoperiod, thermo period, humidity etc.
- Low density of ear-bearing tillers: All the tillers won't flower and only 30-50% tillers possess inflorescence at the time of peak flowering.
- Lodging: Due to prolonged and vigorous vegetative growth lodging of seed crop is a common problem.
- Poor Harvest index: Because of higher biomass production the harvest index is low. Only 2-3% in tropical grasses.
- Lack of seed production technology in fodder and forage crops

**Strategies for increased seed production:**

- Creating awareness to use quality seed of improved varieties.
- Increasing the seed replacement rate from the present 2-3% to at least 20%.
- Seed chain should be followed to produce sufficient quantity of certified seed for farmers
- Improvement of seed chain network
- Seed production through farmer participatory approach
- Improvement of proper marketing facilities
- Research to increase the ovule to seed ratio in forages

- Channelizing the existing demand towards entrepreneurship development
- Improved crop management
- Village Seed Banks are to be developed.

**Research Innovations in forage seed technology**

Several innovations of practical importance were made in forage seed technology in recent years for enhancing the seed yield per se (Vijay et al., 2018).

***External application of IAA in guinea grass:*** The germination of guinea grass is very low and is around 20-30% in best quality seed. To enhance the germination 100 ppm IAA was sprayed during anthesis stage. The seed was collected by daily collection method and bulked. The germination of the seeds was studied in the next season in sand medium. The auxin treatment has improved the rate of germination as well as percent germination. Maximum germination (45%) was recorded with 100 ppm dose of IAA compared to control (26%).

***Invitro rooting in Napier Bajra Hybrid:*** A more packing friendly method of NB hybrid rooted slips was developed. The suitable aged stem cuttings with 2-3 nodes were obtained from the Napier Bajra Hybrid plants. The stem cuttings were wrapped in paper towel layers in such a manner that one node is outside and one node is inside the wrapping. The wrapping was done with 6-7 layers of paper towel with 8 stem cuttings per layer. These wrapped stem cuttings were kept at 25°C and 80% RH. The roots were developed in 7-8 days' time making them ready for transplanting in the field. Since the rooted slips were wrapped with paper and tied they can easily be transported to long distances in cartons. It is a less labour intensive with no need of field requirement/ preparation and is free from seasonal conditions. More than 85% rooting in the stem cuttings was observed and all the rooted slips have developed leaves within 10 days and survived in the field successfully after transplanting.

***High density nursery in Napier Bajra Hybrid:*** In Bajra Napier hybrid due to absence of seed setting, rooted slips are the sole method of propagation. In general the rooted slips are collected from the grass tufts containing 5-10 cm long stems with 2-3 nodes and basal roots. The bi-nodal stem cuttings of at

least 20 cm length and proper thickness are most promising material for high-density nursery. The stem cuttings were closely planted in upward direction after a slant basal cut. Within a fortnight, the cuttings started rooting and shooting and by 4 to 5 weeks they are ready for uprooting and transport. Thus, this technique has several advantages viz., ability to produce rooted slips within short notice; reduced labour requirement; easy management such as irrigation, uprooting, counting, loading due to small area and finally original tussocks are saved.

***In vitro maturation studies in guinea grass:*** The guinea grass panicles were collected just at the initiation of anthesis and were dipped in 100-ppm solution of IAA. The mature seed was collected from the panicles after one week by gentle threshing. This technique helped in reducing the field shattering loss as the seed produced can be collected at one place. The invitro maturation also enhanced the seed filling by almost 15-20% in different cultivars.

***Harvesting at Physiological maturity in Berseem:*** Berseem seed productivity is very low in India leading to severe shortage of available quality seed to the forage farmers. Inflorescence shattering at maturity is one of the main reasons for low productivity in Berseem. The studies conducted on berseem has resulted in identification of proper physiologically mature stage at which shattering can be minimized. The morphological indicator that was identified is colour change in the heads from green to brown with green stalks. During harvesting, the intertwined crop should be rolled over into a heap and dried in the field for one week. This management practice enhanced the production by 50% with yield reaching up to 6 q per ha.

These new innovations in forage seed technology will help in enhancing the seed yield of the respective crops. A combination of these methodologies are to be adopted to realize the seed production potential of fodder species.

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# POST-HARVEST HANDLING, PROCESSING AND SAFE STORAGE OF SEEDS

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Seed is first and important input of agricultural production system. Success of all the agricultural technologies and inputs revolve around seed quality. The genetic purity, physical purity, uniformity in seed size and viability are important to determine the quality of seed. The harvested seed lot contains impurities, improper moisture content, immature and damaged seeds. These components of seed lot invite insects and microorganisms during storage. Storage of seed is essential, as it will be utilized in the next sowing season. In light of above facts, it is essential to know the proper seed processing and storage requirements, for each lot.

Generally, in *Kharif* crops like paddy, maize, pigeonpea etc. at the time of harvest, the seed contains frequently higher moisture content than the optimum. Seed from pulpy fruits like tomato, watermelon, muskmelon, cucumber and brinjal have high moisture content at harvest and absorb more during wet extraction. Other vegetable seeds like onion, brassicas, fenugreek and peas have relatively low moisture at harvest. Sometimes during adverse climatic conditions, seed may also have high moisture content. The seed moisture should be reduced to optimum level before processing and storage. The raw vegetable seed coming for processing is having some percentage of undesirable material. Seed is, therefore, processed for the following reasons:

- To removes plant debris like chaff, straw, flower heads and non seed materials like stones, soil clods etc.
- To remove seed of other crop and common/ noxious weed seeds.
- To remove seed which is of an undesirable quality such as damaged, diseased, insect affected, lighter, larger or smaller than the optimum.

Thus, the basic objective seed processing is to achieve maximum physical purity, germination and uniformity of seed size in an economical way. Wide range of seed processing equipment is available to upgrade vegetable seed quality based on the principal involving physical differences between good seed and material or seed to be removed. Seed separation is done on the basis of following principals:

- Relative size
- Relative length
- Relative shape
- Relative weight
- Relative texture
- Colour

**Drying & moisture**

After proper harvesting and threshing, seed lots should be checked properly for moisture content. If there is higher moisture then the drying is necessary. At the time of harvesting, seed contains a lot of moisture. At high moisture contents there is natural higher respiration rate in the seed which causes deterioration. High moisture also promotes the development of insects. For proper storage of the seed, the moisture content should be 8-16% in various crops. Delays in drying, incomplete drying or ineffective drying reduce seed quality and results in losses, quantitative and qualitative or both.

Drying is the process in which water is removed from seed. Two phenomena involved in drying, i.e. first moisture move from the interior of a seed to the surface and second, the surface water is evaporated into the air. There are different drying methods adopted for proper seed drying.

(a) **Field drying or stacking:** In many traditional harvesting systems farmers leave their harvested seed in the field for extended time because they are either waiting for the thresher or for pre-drying. In field it dries by sun light.

(b) **Sun drying:** Sun drying is the traditional method for drying and is still preferred in Asia because of its low cost compared to mechanical drying. It requires little investment and $CO_2$ neutral since it uses the sun as heat source. Sun drying has some limitations like, it is not possible in night and rainy days, any delay leads to excess respiration and fungal growth, temperature control is not possible. For proper sun drying, spread the grains in thin layer, ideally 2-4 cm. The seed needs to be turned or stirred at least once per hour for achieve uniform moisture content. On hot days cover the seed.

(c) **Air drying method:** The air drying method utilizes air as an agent of moisture removal while its psychometric properties acts as cause drying process. The vapor pressure gradient between seed surface moisture and the air can be increased by increasing temperature of air. As the air temperature increases the water holding capacity increases. In general, seed drying temperature should not be higher than $43^0$C. It is also important the higher temperature should be used to dry relatively dry seeds.

(d) **Fixed-Bed Batch dryers :** Fixed-bed batch dryers have rectangular bins plenum chamber underneath (flat bed dryer, box dryer, inclined bed dryer) or circular bins with central duct. The most common fixed-bed batch dryers are flat bed dryers which have a very simple design. Seed laid out on a perforated screen, and dried by forcing air from below. The fan that provides the drying air is usually a simple axial flow fan that is powered by a diesel engine or electric motor. The capacity of the dryer varies from one to ten tons.

(e) **Re-circulating Batch dryers :** Re-circulating batch dryers have been used for a long time in developed countries. The dryer generally has a drying

section and a tempering section, and seed circulates through these sections in order to alternate drying and tempering. At the same time the seeds are mixed which results in minimal moisture variation in the dried grains. Its size and shape occupies only very limited floor space. The continuous mixing of the grain during the drying operation results in a very low moisture content. Automatic controls with automatic shutoff make the dryer virtually fully automatic.

(f) **Continuous flow dryer :** Conventional continuous flow dryers usually consist of either mixing or non-mixing columnar dryers with different systems of air flow with respect to the seed cross flow dryers are of simple design. In the drying zone the seed moves downwards between two perforated metal sheets while the air moves horizontally through the grains. Since the seed is not mixed moisture gradients develop across the bed. They are also less susceptible to clogging than mixed flow dryers.

(g) **Fluidization dryer :** In this method of drying, the products are being dried under fluidized condition in a dryer. The material is fluidized by drying air with sufficiently high velocity to cause suspension. In this process, higher rates of migration take place. Since every surface of the product is in contact with drying air, uniform drying takes place.

(h) **Vacuum drying :** In vacuum drying, the product is placed inside a chamber where the pressure is reduced to produce a vacuum. Since, the total pressure in the chamber is very low. This low partial pressure causes a large partial pressure difference between the water in the product and the surroundings. Drying under vacuum conditions permits drying at a lower temperature.

After achieving accurate seed moisture content, the seed lot forwarded for processing. Before processing, the seed lots should be checked and properly labeled. The machineries should be cleaned thoroughly. After every variety and crop, machines should be cleaned. There are series machines which are used for processing to get good quality of seed. i.e. air screen cleaner and grader, length separator, specific gravity separator and seed treater.

The seed processing operations can be subdivided into four stages:

1. Winnowing
2. Pre- cleaning or scalping
3. Basic cleaning
4. Separation and upgrading

The actual machines used for any seed lot will depend on the variety or commodity to be processed. Each seed lot does not always require all these processes.

**Winnowing**

Dry seed can be further separated from the low density plant debris by winnowing. Lighter chaff blows away with a stream of blowing air leaving behind the denser seed.

*Winnowing equipment:* The classic method of winnowing involves placing seed in a wide basket and tossing the seeds and chaff into the air. The chaff is carried away by the wind. This method works satisfactorily if the seed is heavy and the chaff is fine, and susceptible to being carried by a gentle current of air. A good range of equipment for winnowing is available and can be operated by hand or mechanically.

**Pre- cleaning**

Bulk of trash is to be removed to facilitate the product flow through the processing machines and elevators. It prevents the clogging of feed hoppers on the processing machines, allows more accurate processing, increases the capacity of the cleaning machines and thereby speeds up the processing operations. Two stage scalping is used to remove major coarse impurities. The performance of machine is a function of pitch of screens, vibration rate of screens, air blast volume, feed rate and cleaning arrangement of screens.

The most commonly used pre- cleaning machine used is a scalper. The operation is normally used to rough clean various kind of trash or plant debris from the seed lot. It may also be used to break up seed clusters during processing. The seeds which are relatively smaller fall through a vibrating or rotating sieve. This separation is usually accompanied by an air flow that removes dust, chaff and other materials which are lighter than the seed crop undergoing pre- cleaning.

**Basic cleaning**

The basic cleaning is the main cleaning of a seed lot. Two main principals involved in basic cleaning are screening and pre-cleaning. The air screen machines combine both these features. It has three processing operations: aspiration; by removing light impurity as well as light seed component, scalping; by removing coarse impurity and grading; by removing under sized seed component. It uses a combination of air flow and perforated metal or wire screens to separate seed on the basis of size. Air screen machines have at least two vibrating screens, the upper screen removes impurities larger than the seed while the lower screen separates out any seed or impurities smaller than the optimum seed size of the crop. An aspirating air flow removes impurities and empty seed which are larger than the optimum. Screens used in an air screen machine are constructed in either perforated sheet metal or woven wire mesh on a wooden frame. Metal screen openings are either round, oblong or triangular while openings in a wire mesh screen are either square or rectangular. These screens can be easily changed and almost hundred sizes are available. It is important that appropriate set of screens are available for the

specific crop seed to be cleaned. The recommended seed sizes for different vegetable seed crops are presented below.

**Screen aperture sizes for major field crops seed processing**

| **Screen aperture size in millimeters** | | |
|---|---|---|
| **Crop** | **Top screen** | **Bottom screen** |
| **Paddy** | | |
| Coarse grain/ bold type | 2.8s, 9.0r **(3.2s)*** | 1.85s **(2.1s)*** |
| Medium slender | 2.8s, 9.0r | 1.80s |
| Fine/superfine | 2.8s, 9.0r **(3.2s)*** | 1.70s **(1.8s or 2.1s)*** |
| **Wheat** | | |
| *T. aestivum* | 6.00 r **(5.5r)*** | 1.80s, 2.10 s, 2.30s **(2.3 or 2.4s)*** |
| **Rapeseed & Mustard** | | |
| Mustard | 2.75r, 3.00r, 3.25r **(3.2r)*** | 0.90s, 1.00s, 1.10s, 1.40r **(1.6 or 1.8r)*** |
| **Maize** | | |
| Maize except popcorn | 10.50r, 11.00r **(10.50r)*** | 6.40r, 7.00r **(6.0s or 6.40s+8.00r)*** |

*Screen aperture sizes being used at IARI RS Karnal

**Screen aperture sizes for vegetable seed processing**

| Crop | Screen aperture size (mm) | |
|---|---|---|
| | Top | Bottom |
| Bittergourd, bottlegourd pumpkin | 11.00r | 6.50r |
| Cucumber | 8.00r | 2.00r |
| Muskmelon | 5.00r | 1.00r |
| Ridgegourd, spongegourd | 9.50r | 6.40r |
| Watermelon | 6.00r | 1.80r |
| Brinjal, chilli, tomato | 4.00r | 0.80s/2.10r |
| Okra | 6.00r | 4.30r |
| Methi | 3.25r | 1.20r |
| Spinach (round seeded) | 5.00r | 2.75r |
| Spinach (sharp seeded) | 8.00r | 2.50r |
| Cauliflower | 2.75r | 1.10r |
| Cabbage | 2.75r | 0.90r |
| Onion | 3.80r | 2.00r |
| Carrot | 2.30r | 1.00r |
| Radish | 4.50r | 2.00r |
| Turnip | 1.80r | 1.20r |

*r-denotes screen with round perforations; s-denotes screen with slotted perforation*

The screens are kept clean during operation by trappers or hammer like screen knockers. Some of the newer machines have highly resilient rubber balls that bounce between the screens and help dislodge clinging materials.

**Separation and upgrading**

These processes follow the main cleaning by air screen machine. They are done for one of the following specific purposes:

- To improve value of the seed
- To increase the concentration of seed
- To remove specific weed, deteriorated crop seed or inert contaminant which was not removed during the basic cleaning.
- To remove an appendage of the crop seed

The commonly used machines for special upgrading of seed are described below

***Indented Cylinder/Length Separator:***

The indented cylinder separator is the second most important cleaning machine. The screens of the air screen cleaner separate seeds mainly according to width and thickness, the indented cylinder separates according to length. As the cylinder revolves, it creates centrifugal force, which helps to hold the seed into the indent. The indented cylinder length separator is a rotating, almost horizontal cylinder with a moveable, horizontal separating through mounted inside it. The inside surface of the cylinder has thousands of small, closely spaced, hemispherical indentations. Shorter seeds are lifted out the seed mass, carried to separating edge of the lifting through and dropped there. Large seed left over in the cylinder are discharged out of the cylinder. The size and shape of the indents combine with seed characteristics to cause separation. The machine also reduces presence of objectionable weed seeds. Position of the separating edge and rotation speed is adjustable. The rate of seed and speed of rotation are two major variables determining machine performance. This machine is especially used for separating pieces of cut seed in wheat, stem or stalk from lettuce seed.

***Gravity separator:***

The specific gravity separator removes undesirable seed and inert contaminants that are so similar in size, shape and seed coat characteristics to the crop seed that cannot be removed in any other way. This is the best machine available for upgrading seed quality. For example, deteriorated, mouldy or decayed seed which are usually similar in size and shape to good seed but have a lower specific gravity can be removed by this machine. Insect damaged seed, empty seed or other seeds that have defects that decrease their specific gravity can be separated on this machine. The gravity separator consists of a base, plenum chamber or perforated vibrating deck, a feed hopper and a seed discharge system. Seed are introduced from the feed hopper on the porous metal deck, where the combination of shaking and air flow up through the deck causes them to stratify seed according to specific gravity. Thus heavier particles walk

close to the deck surface and move towards the top of the deck where they ultimately fall off and are collected. The lighter particles tend to float on the air cushion above the heavier seeds following the path of least resistance and drift to the lower end of the deck where they fall off and are discharged. Seeds in medium specific gravity ranges called *middlings* can be collected in the middle area.

***Electronic colour separator:***

Colour separator makes it possible to separate seeds that cannot be separated by any other method. This type of separation may be necessary on occasions when well graded seed lot contains some discoloured seeds which are known to be of lower potential germination or vigour.

***Mist-O-Matic Seed Treator:***

Seed and treatment material dumps are metered separately. The treatment material flows to a rapidly spinning disc mounted under the seed separating cone. The disc atomizes treatment liquid drops into fine mist and sprays this outward to coat seed falling over the cone through the treating chamber. Later on the seed flows through the mixing chamber housing by an auger conveyor. The coating efficiency can be attained to 85-95%, which is significantly higher than slurry type treaters (75-80%). Each processing line has separate treater, differing only with its capacity i.e. 100 kg/h and 1000 kg/h.

Modern vegetable seed processing line should have the following facilities.

- Receiving
- Pre- cleaning- scalper
- Basic cleaning- air screen machines, indented cylinder separator, gravity separator
- Seed treatment equipment
- Packaging machine
- Despatch

**Packaging**

Seeds of different crops, varieties and classes should be packed in varying sizes primarily in accordance with the seed rate or general requirement. Seed packaging comprises of seed weighing, bag filling, labelling and sewing of the bag. Automatic weigher baggers are utilized in bulk seed processing plant. Seed flows from the product tank and is received in the seed hopper mounted over weighing scale. The weighing scale is attached to the bottom of the hopper. The feed gate opens and closes automatically, as the set weight is attained. After filling, the seed bag is placed on the conveyorised bag closer. The label is sewn to the bag by the sewing head of conveyorised bag closer. As per requirement the capacity of the weighing machine is used.

The material for packaging is a major factor in regulating the moisture content of stored seeds. Seed moisture attains equilibrium moisture content with the

relative humidity (RH) of the surrounding air. The moisture content of the seed during storage will either be low or high, depending on the RH of the surrounding air and on the permeability of the packaging material. The nature of the packaging material is important factor in deciding longevity of seed during storage, Vegetable seed should be preferably sealed in polyethylene or aluminum foil lined PE bags for better storability.

**Seed storage**

Once seed is harvested and processed, it is to be stored for a relatively short or long time till its disposal. The storage conditions must be such where there is minimum reduction of germination and quality of seed.

Normally seeds are stored in jute or canvas bags or low-density polythene sheet or in metal, wood or mud containers. Bulk storage of seed is not practiced in India. These bags or containers are either kept in large sheds or concrete rooms (godowns) or any other type of structures under normal (ambient) conditions. If needed, all containers should be repaired before storage. Seems, sharp corners, holes, etc. should be plugged. Old or used bags should be properly cleaned and treated with suitable insecticide. Old bags of a crop variety must not be used for the same crop in the subsequent season to avoid mixing of varieties.

Thorough cleaning of seed processing plant and storage premises should be done religiously and regularly. Before arrival of new harvest, clean all processing and storage structures thoroughly followed by dis-infestation with residual sprays of insecticides at least four weeks in advance. Insecticides and chemicals used for storage are Malathion 5% dust, Methyl pirimiphos 2%, Malathion 50EC, Methyl pirimiphos 50EC, Deltametharin, Vitavax, Thirum, Captan, etc. Web, dirt, debris, spillage, etc. should be collected and burnt or buried to destroy surviving insect-pests. The storage facility should be white-washed once in year. Spillage of seeds during inspection or drawing samples should be avoided. Spilled seed should immediately be removed because. It attracts wandering insects that may infest and lay their eggs. It can also become the cause of mechanical mixing. Hygiene of store is one of the most important aspects for ensuring freedom from insect-pests inside the seed godowns.

The filled seed bags should always be stacked on wooden pallet. This prevents seed from picking up moisture from the floor, facilities proper cleaning and circulation of fumigant or fresh air. Avoid using steel hook for seed bags as it damage bags and causes spilling of seeds. Alley space should be left between seed-stacks and walls for inspection or sampling. Only 50 to 60% space is utilized for proper storage of seeds. It has been observed that seeds of pulses, cereals and vegetables as well as old or new stocks should be stored separately. It helps in better management of their pest complex because different crop seeds are normally attacked by different group of pests and need different treatment. The timely fumigation is very important for healthy seed storage.

***Effect of temperature on seed longevity***

The general effect of temperature on longevity is that longevity increases as temperature decreases. This is true of 'orthodox' seeds: that is most seeds that follow same general "rule of thumb' regarding longevity during the storage life of the seeds. The relationship between temperature and seed longevity is that for each $10^0$ F ($5.6^0$ C) decrease in temperature longevity doubles (Harrington, 1972). This rule applies to seed stored between temperatures of $32^0$ F ($0^0$ C) and $122^0$ F ($50^0$ C) at a constant moisture.

***Effect of seed moisture and humidity on seed longevity***

Seed moisture has a greater effect than temperature on seed longevity. The storage life of the seed is doubled for each 1% reduction in seed moisture content. This rule applies to seed with moisture content between 5 and 13%. Above 13% moisture content, seed storage fungi and increased heating due to respiration causes longevity. Once seed moisture reaches 18 to 20%, increased respiration and activity of microorganisms cause rapid deterioration of seed. For satisfactory medium term storage of vegetable seeds, the storage temperature and RH should not exceed $10^0$ C and 50% respectively. The activity of storage pests are reduced below 50% relative humidity. In case the controlled storage facilities are not available, the seed should be kept in the coolest and driest place possible. Small quantities of vegetable seed may be stored in desiccators using silica gel to remove moisture from the atmosphere.

**Maximum seed moisture content for seeds stored in sealed containers**

| **Vegetable** | **Seed moisture (%)** | **Vegetable** | **Seed moisture (%)** |
|---|---|---|---|
| Bean | 7.0 | Leek | 6.5 |
| Beet | 7.5 | Lettuce | 5.5 |
| Broccoli | 5.0 | Muskmelon | 6.0 |
| Brussels sprouts | 5.0 | Onion | 6.5 |
| Cabbage | 5.0 | Pea | 7.0 |
| Carrot | 7.0 | Pepper | 4.5 |
| Cauliflower | 5.0 | Pumpkin | 6.0 |
| Corn, sweet | 8.0 | Radish | 5.0 |
| Cucumber | 6.0 | Spinach | 8.0 |
| Eggplant | 6.0 | Tomato | 5-5 |
| Kale | 5.0 | Watermelon | 6.5 |

The chart below shows the expected life span of stored seeds under good storage conditions.

**Expected life span of stored vegetable seeds**

| **Vegetable** | **Years Good germ.** | **Years Average lifespan** | **Vegetable** | **Years Good germ.** | **Years Average lifespan** |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Bean | 3 | 10 | Leek | 2 | 9 |
| Beet | 4 | 10 | Lettuce | 6 | 9 |
| Broccoli | 3 | 10 | Muskmelon | 5 | 10 |
| Brussels sprouts | 4 | 10 | Onion | 1 | 7 |
| Cabbage | 4 | 10 | Pea | 3 | 8 |
| Carrot | 3 | 10 | Pepper | 2 | 7 |
| Cauliflower | 4 | 10 | Pumpkin | 4 | 10 |
| Corn, sweet | 2 | 4 | Radish | 5 | 10 |
| Cucumber | 5 | 10 | Spinach | 3 | 7 |
| Eggplant | 4 | 10 | Tomato | 4 | 9 |
| Kale | 4 | 10 | Watermelon | 4 | 10 |

**Minimum Germination and Purity Limits of Field Crops**

| **Crop** | **Minimum limit of** | |
|---|---|---|
| | **Germination (%)** | **Purity (%)** |
| Paddy | 80 | 98 |
| Wheat | 85 | 98 |
| Mustard | 85 | 98 |
| Maize | 90 | 98 |
| Sorghum | 75 | 98 |
| Pearl millet | 75 | 98 |
| Mungbean | 75 | 98 |
| Pigeonpea | 75 | 98 |
| Bengal Gram | 85 | 98 |

**Minimum Germination and Purity Limits of Vegetable Crops**

| **Crop** | **Minimum limit of** | |
|---|---|---|
| | **Germination (%)** | **Purity (%)** |
| **Cucurbits**<br>Ridgegourd, bittergourd, bottlegourd, tinda, spongegourd, muskmelon, watermelon, pumpkin | 60 | 98 |
| **Solanaceous**<br>Brinjal, tomato,<br>chillies, capsicum | <br>70<br>60 | <br>98<br>98 |
| **Peas and beans**<br>Dolichos, frenchbean, peas | 75 | 98 |
| **Sundry vegetables**<br>Bhindi | 65 | 99 |

| | | |
|---|---|---|
| **Bulb crops** | | |
| Onion | 70 | 98 |
| **Cole crops** | | |
| Cauliflower | 65 | 98 |
| cabbage, knoll khol | 70 | 98 |
| **Leafy Vegetables** | | |
| Amaranthus | 70 | 95 |
| Asparagus | 70 | 96 |
| Methi, lettuce | 70 | 98 |
| Spinach beet (Palak) | 60 | 96 |
| **Root crops** | | |
| Carrot, sugar beet | 60 | 96 |
| Radish, turnip | 70 | 98 |

There are large numbers of insects that infest seeds during storage. These insects infest seeds to fulfill their food and shelter requirements, which results into qualitative as well as quantitative loss of the seeds. Indian climates are very favourable for their multiplication and growth throughout the year. Storage pests also move along with germplasm or seeds from one area to another and even to different countries in the world and create problems. Some adult insects are strong fliers and may spread from one place to another or from the source of infestation. The majority of storage insect pests belong to the orders beetles (Coleoptera) and moths (Lepidoptera). Some minor species belong to Pscoptera.

These insects can be categorized as major pest or minor pest according to the loss of seeds/grains caused by them. Similarly on the basis of their feeding behaviour they can be grouped as Internal or primary feeder and External or secondary feeder.

(a) Internal or primary feeders: These insects mostly lay eggs inside or on the seed and spent a part or entire larval and pupal life inside the seed, only to emerge as adults. They inflict significant loss to the seed quality such as germination and vigour. They are also not detectable from outside e.g. rice weevil, pulse beetle, lesser grain borer, angumois moth etc.

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

Damaged seeds result in loss of germination, serious contamination like webbing and ball formation and also inconspicuous deterioration. It also leads to mould development. According to Indian Minimum Seed Certification Standard the permissible limit for damaged seed in the seed lots is as follows:

i. Maize and pulse seeds not more than 1%.
ii. Other crop seeds not more than 0.5%.

In some insects the seed damage initiates at the ripening stage of crops and continues during storage. Major source of infestations is old bags, storage structure, old containers, and cross over infestation.

***Food grain and Seed losses:*** The Ministry of Food Grain and Civil Supplies has put total preventable post harvest losses to a tune of 10%, of which 6% losses are reported due to storage insects and rodent pests. Similarly Kashyap and Dahiya (1999) reported that out of 1.36 lakh samples of 60 crops, 3.43% samples were rejected on account of insect damage (ID) during 89-90 to 93-94. The status of farmer's save seed was more appalling. Only 13.2% samples were free from insect infestation in Haryana. Around 68% of samples were rejected as per Indian Minimum Seed Certification Standard.

Some important primary insect pests infesting stored seed are illustrated below:

| | |
|---|---|
|  Lesser Grain borer (*Rhyzopertha dominica*) |  Rice weevil (*Sitophilus oryzae*) |
|  Pulse beetle (*Callosobruchus sp*) |  Angoumois grain moth (*Sitotroga cerealella*) |

***Pest control in stored seeds and food grain:*** Various techniques are used to control insect pests in stored produce-from sunning and smoking on the traditional farm to irradiation in large-scale bulk handling. The proven techniques suitable for use in small- and medium-scale storage under tropical conditions are discussed here.

The technique must achieve one of the goals such as,

(a) Economics (the value of the product, relative to the cost of technique and labour);

(b) Pest problems (such as occurrence and resistance level); and

(c) Techniques within the farming system or through the availability of new products.

Therefore, it is important to consider both economics and technical specifications for effectiveness against target pests and hazards to farmer and consumer.

**Insect Pest control techniques:**

***(A). Sanitation:*** It is very important to reduce the initial pest population and prevent development of any insect pests in the grain or seed. Before bringing fresh material into the store, the following steps are necessary to adopt.

- Remove infested material. Do not mix new produce with old;
- The old material that must be kept should be thoroughly fumigated.

- Clean the storage structure.
  - Brush away all traces of spilled grain, dust, etc.
  - Remove dust from handling equipment and machinery.
  - Disinfect sacks and baskets by sunning or chemical treatment.
  - Be aware of the specifications for chemical treatments.
  - Large structures usually require chemical treatment
  - Small rural structures can be cleaned by using smoke, and making use of the sun and rain-after some time insects will usually leave a clean empty structure.

Take control measures early (at pre-harvest stage) to prevent infestation of crops maturing in the field.

There are some useful general characteristics in the following crop varieties that help grain/seed to escape insect attack:

(a) Maize: good husk cover can reduce field infestation, and storing in the husk reduces the rate of pest increase;

(b) Sorghum: varieties where the glumes cover the grain tend to be more resistant before threshing;

(c) Rice: paddy rice is considerably more resistant to pests than milled rice;

(d) Cowpeas: intact dry pods provide better protection against bruchids; if fumigation or airtight storage is impracticable, cowpeas are better stored un-threshed;

(e) Cereals: grain hardness affects resistance.

***(B) Hermetic or sealed storage.*** In airtight conditions, reduced oxygen and increased carbon dioxide arrest insect and mould development thereby keep produce free from insect infestation.

- Grain for human consumption or seed must be dried for better storage. In damp grain bacterial and enzymatic action will continue, causing discolouration and loss of viability.
- Bagged material must be protected; if the seal is broken (by insects, rodents or careless handling) the grain is unprotected and unventilated, and losses may be severe.
- A method that has been found satisfactory where in threshed cowpeas is stored in sealed plastic bags with cotton liners; the cotton prevents emerging insects from perforating the plastic bag.

***(C) Chemical control:*** Methods of using insecticides on stored products are:

- Dusting
- Spraying
- Fumigation

Insecticides usually show some degree of toxicity to humans, domestic animals, poultry, etc. and must be used only for seed with caution. Be sure to:

- read the manufacturers' instructions;

- choose a chemical with low toxicity to seed and warm blooded animals and birds;
- stay within the recommended dosage; and
- Insecticides are usually specific, and do not kill all insects and mites; choose a chemical approved for use in stores and/or on stored products that has either a "broad spectrum" or specific toxicity to moths and beetles. Mites may require special treatment.

Insecticides tend to lose their persistence, or effectiveness and also may affect germinability of seed. Therefore, it should be used with great caution and as per recommendation. The following factors affect insecticide's toxicity:

- Type of Insecticide and Its Dosages
- Storage Conditions
- High Humidity
- High Temperatures
- Sunlight
- Time

***(D) Fumigation***. Chemicals used to attack insects through their respiratory system are known as fumigants.

Fumigants may be formulated as:

| | |
|---|---|
| 1. Liquids<br>▪ Carbon Bisulphide<br>▪ Carbon Tetrachloride<br>▪ Ethylene Dichloride<br>▪ Ethylene Dibromide | 2. Gases<br>▪ Hydrogen Cyanide<br>▪ Methyl Bromide<br>▪ Phosphine |

The concentration of a fumigant is measured in mg/l of space occupied. Some of the most commonly used fumigants are:

(1) Methyl bromide is an excellent fumigant, but it has no smell and is very poisonous. It can therefore only be used by trained teams. It is now banned for commercial use and not available.

(2) Phosphine is an excellent fumigant and fairly easy to use. It is used as a mixture of aluminium phosphide and ammonium carbamate. These are stable if kept in sealed containers, but when exposed to the air they take up water and release phosphine, ammonia and carbon dioxide. Phosphine normally contains impurities which make it spontaneously inflammable, but in the presence of ammonia and carbon dioxide it is safe. The chemicals are formulated so that there are about 30 minutes available to distribute the mixture before the gas is released. The gas has a strong and unpleasant garlic type smell and is therefore easy to detect. Phosphine is the only fumigant that will not interfere with germination if the grain is to be used for seed. The others may affect germination if exposure to the fumigant is excessive or repeated.

Fumigation in practice involves consideration of the scale of the operation, and safety.

In larger-scale fumigation, phosphine fumigation may be carried out using formulations which consist of tablets, pellets or powder in envelopes. The makers issue instructions for the quantities to be used. Sacks of seeds may be fumigated under plastic sheeting: It is stacked on a sheet of plastic, covered with another sheet, and after the fumigant is inserted the top and bottom sheets are rolled together. Sandbags are placed on the roll to give an airtight seal.

Fumigation Methods include fumigating produce in containers, or fumigating surfaces. The produce is placed in drums, plastic bags under tarpaulins or plastic sheets. After addition of the chemical, the produce must be kept in airtight conditions for at least three days for Phostoxin or about one day for EDB, depending on the doses applied. For fumigation of stacks in warehouses, it is necessary to spray the roof and walls simultaneously to prevent re-infestation. Grains must be protected from subsequent re-infestation.

Safety must be a primary consideration. All fumigants can kill people as well as insects, and some may cause serious disorders in humans who are exposed to low concentrations over a long time. Consequently, stocks of fumigant should not be kept in offices or stores where people are working. Accidents can happen, so two people should always be present when fumigating. Fumigation should only be carried out by trained staff operating under proper supervision.

***(E) Insecticidal sprays***. The method of using sprays is to allow 10-15 ppm active ingredient (ai), contained in the minimum of water necessary to give an even coverage (about 0.3-2 l/tonne, depending on the applicator). Such a small quantity of water will not cause moulding. The insecticide can be applied with a small domestic applicator (Shell/ox-type), but a knapsack sprayer reduces labour requirements.

Application of sprays differs with the type of storage facility used. In warehouses, the following procedures are used.

(a) Bagged produce. Each layer of bags is sprayed as the stack is built; this should give protection for several months, but in case of re-infestation, the stack should be fumigated and re-sprayed for effective penetration.

(b) Space spraying. A non-persistent insecticide is sprayed to kill the adults of flying insects, especially warehouse moths; used in conjunction with fumigation under sheets.

(c) Fogging. For the same purpose, an electric applicator delivers very fine droplets, which hang in the air, maximizing effectiveness.

(d) Surface treatment. A persistent insecticide is sprayed on walls, roof and floor of the storage structure.

Among suitable chemicals are the synthetic pyrethroids such as deltamethrin, usually used for space-spraying and/or control of the larger grain borer; dichlorvos (DDVP) for automatic fogging (Note: this is highly toxic to mammals);

and malathion or pirimiphosmethyl for general use (since it costs less and has lower toxicity).

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# VARIETAL MAINTENANCE AND PRACTICAL ASPECTS IN SEED PRODUCTION OF VEGETABLE CROPS

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Seed is the basic starting point of most of the vegetable crops, although some are propagated vegetatively from cuttings or tubers. Therefore, quality seed is one of the important inputs for increasing vegetable productivity. It is essential that seed of highest possible quality may be made available to the farmers. However, at the time of variety release a small quantity of seed is available with the breeder. The relatively small amount of seed of improved cultivar needs to be multiplied and made available to farmers as quickly as possible. During seed multiplication, varietal purity and identity needs to be maintained. Each multiplication cycle starts from the 'breeder seed'. If the breeder seed is not of high purity, the contaminants present get multiplied several times in the succeeding generations of foundation and certified seed production. The presence of contaminants may even lead to complete loss of the improved features of the cultivar. Prevention of contamination is at the heart of a successful breeder seed production programme.

**Variety Maintenance**

Maintenance of varieties means preservation of the variety in its original form, or even improving it while not losing its characteristic features or its individuality. During seed multiplication process, several factors may reduce the genetic purity of seed. The maintenance of genetic purity is largely dependent on the genetic makeup of the cultivar. The diversity of morphological types is normally greater in predominantly cross pollinated crops like brassicas, cucurbits, onion etc than self- pollinated crops like garden pea, vegetable cowpea, fenugreek, tomatoes, etc. Genetic variation may appear within a seed stock for a number of reasons including mechanical contamination, hybrids resulting from undesirable pollination, recombination, residual segregation, and mutation. These factors ensure that no cultivar is likely to retain the precise frequencies established by the breeder without continuous intervention through maintenance breeding techniques. The occurrence of contaminants during seed multiplication process occurs due to:

1. **Out-crossing**: Out-crossing vegetable crops such as onion, brassicas, cucubits, onion have substantial outcrossing percentage and even self pollinating crops like common beans show a low percentage of out crossing. In each generation many new genetic combinations appear due to uncontrolled open-pollination. These new genotypes recombine and segregate into a range of new genotypes, thereby enlarging the genetic variations within a cultivar.

2. **Residual segregation**: Some characters have not been selected during breeding or in subsequent purification and production may remain in a population in a range of states. Exposure to new environments may allow expression of states of selected characters not detected previously. For example in red beet (*Beta vulgaris* L.) maintenance of bolting resistance is strongly influenced by environmental conditions. To maintain such cultivars, a strong selection pressure for that (bolting) attribute is required.
3. **Volunteer plants**: Volunteer plants may arise from vegetative pieces or dormant seed remaining from previous crop grown on the same field. If the volunteer plants are of the same crop to which the cultivar to be maintained belongs will enlarge the genetic variation once harvested together - For example volunteer plants are seen in Indian spinach beet, tomato etc.
4. **Mechanical mixture**: Mechanical mixing with seed from another cultivar of the same crop may occur during sowing, harvesting, threshing, cleaning, storage, bagging, etc.
5. **Mutation**: During maintenance and production spontaneous mutations do occur and may reduce the agronomic value of the cultivars. The mutation rate is generally low but increases significantly after long periods of seed storage. Since mutations are usually micro mutations and recessive, they are often difficult to detect. However mutations are probably the least important degrading factor contributing to genetic contamination.
6. **Seed borne pathogens**: Pathological contaminants occur due to infection with seed borne diseases, which are exclusively transmitted through seeds. Pathogens like viruses in tuber crops or anthracnose in beans can spread rapidly, if not contained. Pathological contaminants result in loss of yield and quality of cultivars and also gradual loss of tolerance of the cultivar to specific seed borne diseases.

The above mentioned factors collectively result in genetic variation and these changes accumulate over the years resulting in loss of varietal identity and genetic gain.

## Maintenance of a cultivar

Once a cultivar is released for cultivation, the breeder usually supplies a small quantity of seed for further multiplication and maintenance. The responsibility of breeder seed production centre is to produce breeder seed and varietal maintenance. In order to release seed of an improved cultivar to farmers, it has to be multiplied. Each multiplication cycle has to start from its basic seed stock, 'Nucleus Seed'.

Our basic objective of cultivar maintenance is to maintain the purity and identity of a cultivar. The maintenance procedures are in fact the extension of the normal breeding process. The difference is that during maintenance

breeding, selection process is relatively mild and our aim is not improvement but to keep the identity unchanged. Selection should maintain the plant type, its uniformity and freedom from diseases. The maintenance procedures for self pollinating vegetable crops like garden pea, cowpea, fenugreek, tomato etc with a substantial amount of out-crossing are slightly different from cross-pollinating crops. In cross pollinating crops like cauliflower, cabbage, onion, carrot, etc the important characters are assessed before flowering. The fields where plants and progenies are to be assessed should be uniform. Essentially these should be grown under optimal growing conditions.

## Cultivar maintenance in self-pollinating vegetable crops

***Plant to row method****:* The maintenance procedure starts with a small plot raised from the parental material received from the breeder or uniform seed multiplication field in case of established cultivars. A fair number (300-500) of healthy plants typical of the cultivar are selected and marked for progeny testing. The seeds of the marked *true- to- type* plants are harvested separately. The seeds of each plant are planted in a 3m long progeny row. These progeny rows are assessed critically several times during the growing season. Progeny rows that deviate in one or other characteristics are discarded and entire plant progeny rows is rejected. The plant progenies that are uniform and true to type are selected and bulked together as nucleus seed stage-I. This nucleus seed is used for planting larger breeder seed plots. If the breeder seed requirement of a particular cultivar is more, then another cycle of nucleus seed production is followed.

In this case the *true- to- type* individual plant progenies are harvested and thrashed separately. Seed of each selected plant- row progeny is now sown in a small plot called plant- row- progeny plot. The second cycle of nucleus seed production provides another opportunity to eliminate any plant row progeny showing segregation or off-type plants. The plots with required uniformity and plant type are bulked together to produce nucleus seed stage-II. The product of this second cycle of progeny testing is sufficient to meet the higher requirements of breeder seed particularly in garden pea, vegetable cowpea, etc.


**Plate 1:** Nucleus seed production in garden pea

## Cultivar maintenance in cross-pollinating vegetable crops

The risk of genetic contamination through pollen of other cultivars is more in cross pollinating crops and therefore, large isolations are necessary. Cross pollinating cultivars cannot be maintained unchanged for a prolonged period unless proper and systematic maintenance selection procedures are followed. In these cultivars plant selection is of vital importance because of the inherent genetic variation within the crop. However, too stringent selection can shift the cultivar type as much that a different type is produced if reference to original description is not maintained. The methods used for maintenance of such cultivars are

***Negative mass selection*:** Part of the basic seed field is given special attention. The undesirable premature bolters and diseased plants are removed. Any doubtful plant not conforming to cultivar description should also be removed. Isolations from related crops should be maintained. The left over plants are harvested and bulked together and is utilized for breeder seed production. In this method, there are chances of cross contamination from off-type pollen grains from undesirable plants. In case of melons nucleus seed is produced by mass selection based on fruit quality in respect to fruit shape, flesh colour and TSS. In watermelon fruit scoring >10% TSS are selected where as in muskmelon fruits showing TSS> 12% are selected for nucleus seed.

**Plate 2:** Nucleus seed production in melons**.**

***Rest seed method*:** From the basic seed plot, select 300-500 true-to-type plants based on the cultivar characteristics. The selected plants are harvested and threshed individually. Next year a small part of each progeny (about 200 best plants) is planted in rows and the remaining seed of each progeny is stored. Each plant-row progeny is critically examined for cultivar characteristics and rows showing off type plants are marked and rejected. The remnant seed of the true-to-type plant rows is bulked to form the nucleus seed and is used to raise the breeder seed plots next year. All off-type plants are removed from the progeny rows before flowing until harvest. Again 300-500 best plants are selected for next maintenance cycle. Repeated maintenance, selection results in a continued improvement over time provided the number of progenies is kept fairly large.


**Plate 3:** Nucleus seed production in okra**.**

**Vegetable Seed Production Technology**

Quality of seed includes; genetic purity, physical purity, germination potential and freedom from pest and diseases. To produce seed with all these quality attributes, a systematic seed production is must. During the seed multiplication process, the following points are to be kept in mind for obtaining high yields of quality seed with low cost of production.

***Environmental Requirements***

Vegetable crops for seed production should be grown under best possible conditions. Best seed yield and quality is obtained when crop is grown in correct season and in an area where the crops' reaction to appropriate environment can be observed. The regions with abundant sunshine should be selected for seed production. The flowering in many crops is controlled by the light duration (Photoperiodism). For example lettuce and spinach require long day conditions for flowering and seed setting. Some species require a low temperature stimulus to initiate flowering (vernalization). Many temperate vegetables like cabbage, cauliflower, beet root, European type radish and carrot also require vernalization. Avoid areas of high rainfall as it may reduce seed viability and increase need for artificial drying. Excessive wind will increase water loss, prevent activity of pollinators, carry wind borne pollen over long distances and increase seed shattering.

***Land Requirements***

The field selected for raising a vegetable seed crop should be free from 'volunteer' plants. Volunteers mean the plants originating from the seed/plant material of the previous market or seed crop. In vegetables volunteer plants are seen in palak, tomato etc. The land should be leveled with proper drainage and should have sufficient organic matter. The cultural operations of vegetables for seed production are similar to the operation on market crop but seed crop is to

be maintained beyond the stage of commercial market harvest. Therefore, the control of diseases, pests and weeds should be continued over a longer period.

***Pollination Requirement***

Vegetable crops like tomato, garden pea, fenugreem, cowpea are self pollinated and majority of other vegetable crops like okra chillies, cucurbits, brassicas are cross pollinated.

**Table 1.** Natural cross-pollination (NCP) and pollination agents in vegetable crops

| Crop | NCP (%) | Pollination agent |
|---|---|---|
| Tomato | 0.00-5.00 | Honey bees/ solitary bees |
| Potato | 0.00-20.00 | Bumble bees |
| Brinjal | 0.70-15.00 | Insects |
| Capsicum | 7.00-37.00 | Honey bees/ insects |
| Carrot | 97.6-98.90 | Insects / bees |
| Radish | Highly CP | Bumble bees/ honey bees |
| Cabbage | 73.0 | Honey bees/ bumble bees |
| Cauliflower | 40.0-50.0 | Honey bees/ bumble bees |
| Onion | 95.0-100.0 | Insects |
| Muskmelon | 85.0-95.0 | Honey bees |
| Cucumber | 65.0-70.0 | Honey bees/ solitary bees |

The natural insect population is normally sufficient under open conditions to ensure satisfactory pollination but high plant population grown for seed in a concentrated area, there is a possibility that natural insect population may be insufficient to ensure proper seed set. Therefore, the introduction of supplementary bee hives will improve pollination and seed set. However, care must be taken to ensure that pest protection chemicals are not used in a way to harm useful pollinating insects. Spray of chemicals should be avoided at peak pollination insect activity and should be done in the late afternoon.

***Isolation Requirements***

Satisfactory isolation of seed crop helps to maintain purity in three ways:

a) Cross-pollination does not occur between cross compatible crops
b) During harvesting seeds of different varieties of same crop are not mixed
c) The transmission of pest and diseases from alternative host crop are minimized

Proper isolation is thus essential to maintain genetic purity and health of a variety. Isolation between cross compatible varieties is achieved as follows.

***Isolation by time:*** Isolation by time will allow seed of different varieties of the same crop to be produced at the same station each year. If the season is too long enough to allow two production cycles of the cross compatible crops then they too are isolated by time. For example, early and mid maturity group of cauliflower grown for seed production can be isolated by time.

***Isolation by distance:*** The mode of pollination is related to the degree of isolation necessary. In case of self-pollinated varieties the isolation distance is relatively short but, in case of cross-pollinated varieties the distance from other variety should be relatively wide. The isolation distance also depends on the direction of insect flight (in case of insect pollinated varieties) or the direction of winds (in case of wind-pollinated varieties).

**Table 2:** Minimum isolation requirements of vegetable seed crops

| **Crop** | **Minimum isolation distance (m)** | |
|---|---|---|
| | **Foundation** | **Certified** |
| Brinjal | 200 | 100 |
| Fenugreem | 10 | 5 |
| Tomato, beans, dolichos, cowpea | 50 | 25 |
| Cauliflower, cabbage, beet raidsh, turnip | 1600 | 1000 |
| Carrot | 1000 | 500 |
| Bhindi, chilli, capsicum | 400 | 200 |
| Bottlegourd, muskmelon, watermelon, spongegourd, bittergourd, pumpkin | 1000 | 500 |

***Roguing***

The vegetable varieties, which are produced for seed show genetic change over several generations. It is, therefore, necessary to exert control and keep the natural variation within the acceptable limits. This is achieved by inspecting the crops at various growth stages and removing individual plant which do not confirm to the defined limits of that variety. Thus rouging is a technique that is used in seed production to maintain genetic purity of the variety. Rogues or off-types may occur in a crop due to any of the following reasons.

- The diversity of the morphological types within a crop may be wide. This tendency is greater in predominantly cross-pollinated (e.g. cauliflower, cabbage, cucurbits and onion) than self-pollinated (e.g. peas, tomato, fenugreek) crops. This is why varieties of self-pollinated crops are generally more uniform and stable than varieties of cross-pollinated crops.
- The seeds that result from cross-pollination between the crop for seed production and other compatible varieties or wild plants. These are not always identified in the first generation and show up in the second.
- Some plants may display deviation from the normal type due to mutation.
- Seeds of other varieties may have been accidentally mixed in the seed stock during its production, processing or admixture via seed drill.
- Volunteer plants may arise from vegetative pieces or dormant seed of the previous crop grown in the same field.

It is always easier to conduct intensive rouging in breeder seed plots than in large commercial seed production plots. To obtain maximum benefits from rouging operation, we should follow the below mentioned practical points.

- The crop should be grown in such a way that plants can be seen individually. Paired row system of planting may be followed so that it is easy to walk between rows. This shall facilitate detection of dwarf undesirable plants.
- Walk systematically through the crop so that each plant is seen. Remove the whole off-type. Do not simply remove the fruits showing undesirable character because the remaining flowers on the off-type plant will still contribute to the material in the n ext generation.
- Inspect the crop with the sun behind you as it is difficult to examine plants with the sun on your eyes.
- Do not delay field inspection. The undesirable plants should be removed before flowering as far as is possible. Remove cross-compatible weeds and wild relatives. Remove all diseased plants and related infected weeds also.

Variety description based on morphological characters like leaf shape, flower colour, fruit shape and colour generally from a good basis of rouging but some characters like leaf colour, plant height, earliness of flower are affected by environment.

**Plate 4** Roguing operation and Joint monitoring inspection in vegetable seed crops.

***Harvesting, threshing and seed extraction***

The best time of harvesting vegetable seed crops is at a stage when the highest yield of best quality seed will be obtained. Seed has to be extracted from dry seed heads, or from dry fruits or from fruits in which the seeds are wet at the time of extraction. Threshing can be done by hand, animal or machines. Care should be taken while transportation of material from the field to threshing floor. Both the trolley and the threshing floor should be clean from the seed/ plant parts of the other varieties of the same crop or weeds to avoid admixture at this stage. Threshing machines must be used with care in case of

vegetables. They should be run at a reduced speed to avoid mechanical damage to the seed. Threshing machines should be properly cleaned to avoid admixture.

***Seed drying***

At the time of harvest, the seed contains frequently higher moisture content than the optimum for better germination and storability of seed. Seed from pulpy fruits like tomato, watermelon, muskmelon, cucumber and brinjal have high moisture content have high moisture content at harvest and absorb more during wet extraction. Other vegetable seeds like onion, brassicas, fenugreem and peas have relatively low moisture at harvest. Sometimes dur to adverse climatic conditions, seed may also have high moisture content. The seed moisture should be reduced to optimum level before storage. For ambient storage seed moisture should be kept under 9-12% and for sealed storage it should be 6-8%. Natural and artificial methods are used in vegetable seed drying.

***Seed Quality control***

Seed quality reflects the overall value of the seed for intended purpose. Poor quality seed leads to loss of money and potential crop. Seed quality consists of physical purity, germination, potential, genetic purity and seed health.

**Table 3:** Minimum Germination and Purity Limits of Vegetable Crops

| **Crop** | **Minimum limit of** | |
|---|---|---|
| | **Germination (%)** | **Purity (%)** |
| **Cucurbits** | | |
| Ridgegourd, bittergourd, bottlegourd, tinda, spongegourd, muskmelon, watermelon, pumpkin | 60 | 98 |
| **Solanaceous** | | |
| Brinjal, tomato, | 70 | 98 |
| chillies, capsicum | 60 | 98 |
| **Peas and beans** | | |
| Dolichos, frenchbean, peas | 75 | 98 |
| **Sundry vegetables** | | |
| Bhindi | 65 | 99 |
| **Bulb crops** | | |
| Onion | 70 | 98 |
| **Cole crops** | | |
| Cauliflower | 65 | 98 |
| cabbage, knoll khol | 70 | 98 |
| **Leafy Vegetables** | | |
| Amaranthus | 70 | 95 |
| Asparagus | 70 | 96 |

| | | |
|---|---|---|
| Methi, lettuce | 70 | 98 |
| Spinach beet (Palak) | 60 | 96 |
| **Root crops** | | |
| Carrot, sugarbeat | 60 | 96 |
| Radish, turnip | 70 | 98 |

***Genetic Purity:***

Trueness to type can be assessed by grow-out tests. In addition, biochemical and cytological methods can also be used to ascertain the variety purity. Seed Certification scheme can effectively control genetic purity of seed crops.

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# VARIETAL MAINTENANCE AND QUALITY SEED PRODUCTION IN RICE

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Maintenance breeding is the backbone of any quality seed production system. A new improved variety is a product of consistent and time consuming breeding efforts. These breeding gains can only be realized, if genetically pure seed in adequate quantities is made available to farmers. Without proper maintenance breeding, varieties become defunct, however, excellent these may be in performance (Fig. 1).

**Maintenance breeding**: Maintenance breeding is a key technique for purification and stabilization of pipeline / released varieties. The varietal maintenance work on basmati and non-basmati rice varieties, undertaken at IARI Regional Station, Karnal has made Pusa bred basmati and non-basmati rice varieties very popular among farmers and in export markets (basmati rices).

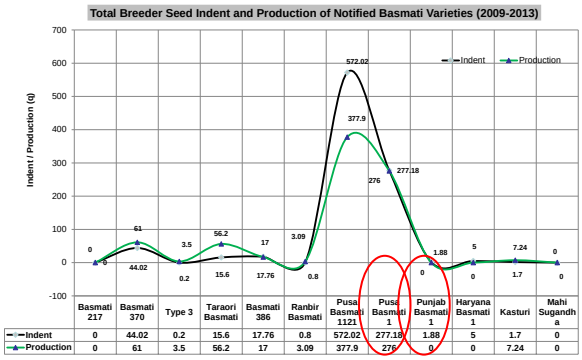


**Fig. 1: Maintenance Breeding: reason behind spread & popularity of Pusa bred basmati varieties**

Initially eleven varieties (six traditional and five evolved) were notified as Basmati varieties under Seeds Act (1966). The comparative appraisal (Fig. 1) of breeder seed indents and production of these eleven basmati varieties along with Pusa Basmati 1121, clearly demonstrates the lead role of Pusa bred basmati varieties. The main reason for popularity and spread of Pusa Basmati varieties is the strong varietal maintenance breeding component underpinning the spread of genetically pure seed of these varieties. It is quite possible that if remaining basmati varieties in Figure 1 had been given adequate maintenance breeding support by respective institutes / SAUs these varieties could have also made a significant dent at national level. India exported around 4.04 million tonnes of basmati rices valued at Rs. 22,718 crores during 2015-

**Pusa Basmati 1: An outstanding example of varietal maintenance (Notification year 1989)**

16. (DGCIS Annual Export figures). Major chunk of these exports is contributed by Pusa basmati varieties. Thus, appropriate varietal maintenance not only enhanced consumer acceptability and export earnings (Pusa Basmati 1121, Pusa Basmati 1) but also increased the productive life a variety (Pusa 44, Pusa Basmati 1).

In Indian generation system of seed multiplication there are three recognized classes of seed i.e. breeder, foundation and certified seed. Seed supply chain in India follows a three to four tier system of multiplication i.e. BS→ FS→ FS/CS→ CS. The seed multiplication cycle starts from the 'breeder seed', which is dependent upon availability of high quality nucleus seed (a product of varietal maintenance). If breeder seed is not of high genetical purity, contaminants present get multiplied several times in succeeding generations. Presence of contaminants may even lead to complete loss of desirable traits of variety, for which it was bred. Therefore, prevention of genetic deterioration and contamination is critical for a successful seed production programme. This can be achieved by putting in place an effective system of varietal maintenance and nucleus seed production. In self-pollinated crops, genetic deterioration may be expected to reach a significant proportion by the fourth generation onwards. This genetic deterioration may be caused by residual segregation, spontaneous mutations, natural out crossing or mechanical admixtures. To prevent genetic deterioration / contamination and to enhance the productive life of a variety, only tool available is maintenance breeding. Varietal maintenance of basmati rice and non-basmati varieties at Karnal, is a prime example of effectiveness of this approach in enhancing the productive life of these varieties (Table 1). The best approach for varietal purification, maintenance and nucleus seed production of rice is Panicle to Row method, which is briefly described below.


**PB 1637: Varietal maintenance plots of newly notified variety with blast resistance genes**


**Cooking Test: An integral part of varietal maintenance of Basmati rices varietiesvarietiesVarietal maintenance plots of a variety with cooking quality par excellence**

- Around 350 "true-to-type" single panicles are selected
- Selected panicles are threshed separately and are critically screened for seed characteristics
- In case of basmati varieties, a part of seed is also subjected to cooking analysis

- Seed of panicles not conforming to seed characteristics or not performing well in cooking test are rejected
- Seed of remaining 200-250 panicles are raised in panicle-rows. At Karnal, we prefer to raise paired rows from a single panicle
- Rigorous screening of panicle rows is done for their stable diagnostic characteristics at different crop growth stages
- Panicle rows not conforming to "true to type" plant type or showing off types are completely removed as and when observed


**PB 6: Varietal maintenance plots of a variety with cooking quality par excellence**

- Remaining selected panicle rows are harvested and threshed individually and seed of each individual panicle row is critically examined
- Eventually seed of selected true to type panicle rows are bulked to obtain genetically pure high quality nucleus seed

**Table 1**: Improved Varieties of Basmati and non-Basmati Rice undergoing Maintenance Breeding at IARI Regional Station, Karnal

| Variety | Notification | |
|---|---|---|
| | No. | Year |
| Pusa Basmati 1 | S.O. 915 (E) | 06.11.1989 |
| Pusa 44 | S.O. 636 (E) | 02.09.1994 |
| Pusa Sugandh 5 | S.O. 122 (E) | 20.02.2005 |
| Improved PB 1 | S.O. 1178 (E) | 20.07.2007 |
| Pusa Basmati 1121 | S.O. 2547(E) | 29.10.2008* |
| Pusa Basmati 6 | S.O. 733 (E) | 01.04.2010 |
| Pusa Basmati 1509 | S.O. 2817(E) | 19.09.2013 |
| Pusa 6 (Pusa 1612) | S.O. 2817(E) | 19.09.2013 |
| Pusa 1592 | S.O. 1556 (E) | 11.06.2015 |
| Pusa Basmati 1609 | S.O. 2680(E) | 01.10.2015 |
| Pusa Basmati 1637¥ | S.O. 3540 (E) | 22.11.2016 |

*earlier notified as Pusa 1121 (S.O. 1566 (E) dated 05-11-2005)
¥newly notified variety

Integration of cooking and grain quality test and screening for disease resistance genes (in NILs) in the maintenance breeding programme itself has significantly enhanced the consumer preference and longevity of these varieties. The focus is stable diagnostic characteristics coupled with cooking and quality testing (Kernel length, elongation ratio, aroma etc.), keeping in view the guidelines of Govt. of India for delineating varieties qualifying as Basmati varieties.

**Seed Production:**

Pusa Basmati 1509: Seed Production Plot

Quality seed are of high species and cultivar purity (genetic purity), analytical (physical) purity, high germination capacity and vigor; uniform in size; free from weed seed; free from seed borne diseases; and with low moisture content.

Seed quality as an explicit paradigm is a recent phenomenon, and is coterminous to development of seed technology as an independent interdisciplinary science. The crux of transformation from traditional to modern agriculture was the proper understanding of the concept of "Seed Quality" & the critical distinction between "grain" and "seed". The quality seed or improved seed has two distinct sources of improvement (i) genetic information contained in seed (i.e. improved variety) (ii) physical and physiological attributes of seed lot (i.e. purity and germination). Genetic quality is the ultimate determinant of performance, but if physical and physiological quality is poor, the benefits of genetic potential can-not be realized.

**Seed Production Technology:**

Seed production is different from crop production. Quality seed production of rice should be planned systematically so as to minimize the chances of genetic contamination as well as mechanical admixtures. The important protocols of rice seed production based on IMSCS (Indian Minimum Seed Certification Standards, 2013) are described below.

**(i) Application and amplification of general seed certification standards**:

It is important for seed producers/farmers to be aware of the prescribed seed certification standards (general and specific) of the specific crop for which they are undertaking seed production programme (e.g. here in this case Rice).

**(ii) Land requirement:**


PB 1637 Breeder Seed Plot: Paradigm shift in Basmati breeding with focus on incorporating disease resistance

Land to be used for seed production programme should be free from volunteer plants (self-sown seeds). If land requirement is not checked then the resultant seed crop may have problems of ODVs (Other distinguishable varieties) and / or physical admixture because of volunteer plants (Table 3). The

quantification of ODVs is must and not voluntary for certified seed (where standards are available). Labeled seed is not tested for this seed quality attribute.

**(iii) Field inspections:**

Field inspections are made in the field on the standing crop and are meant to verify those factors which can cause irreversible damage to the genetic purity or seed health. Field inspections also check that the seed being produced is of the designated variety and has not been contaminated genetically or physically beyond certain specified limits. Minimum two field inspections are undertaken in rice, first during flowering and second before harvesting.

**(iv) Field standards:**

Field standards have two components: (i) general requirements dealing with isolation (ii) specific requirements indicating maximum permitted off types. Field standards have critical impact on the genetic purity of the seed crop (Table 2).

**Table 2:** Field Standards

| **(A) General Requirements:** Isolation distance | **Minimum distance (m)** | |
|---|---|---|
| | **FS** | **CS** |
| Fields of other varieties | 3 | 3 |
| Fields of other varieties not conforming to varietal purity requirements for certification | 3 | 3 |
| **(B) Specific requirements** | **Maximum permitted (%)*** | |
| | **FS** | **CS** |
| Off types | 0.05 | 0.20 |
| ** Objectionable weed plants | 0.01 | 0.02 |

*Standards for Off-types and objectionable weeds shall be met at the final inspection.

**Objectionable weed shall be: Wild rice (Oryza sativa L. var. fatua Prain) (Syn. O. sativa L.f. spontanea Rosch.)

**Roguing: Removal of off types and unwanted plants**


**Pusa Basmati 1121: A wonder variety of basmati rice**

Quality of seed crop is hampered by (i) Off types (ii) Inseparable other crop plants (iii) Objectionable weed plants (iv) diseased plants. The removal of these unwanted plants is called rogueing. Roguing is a critical component of management of genetic purity of a crop.

**(v) Seed standards:**

After seed crop harvesting, the seed lots are checked against prescribed standards, thereby ensuring seed quality to end-users (Table 3). Thus, if seed lots meet prescribed field and seed standards, it can be described as quality seed.

If the aforesaid points are taken into considerations, then the seed producers can themselves produce their own genetically pure high quality seed of paddy varieties.

**Table 3 :** Seed Standards

| **Factor** | **FS** | **CS** |
|---|---|---|
| Pure seed (%), ↓ | 98.0 | 98.0 |
| Inert matter (%), ↑ | 2.0 | 2.0 |
| Huskless seeds (%), ↑ | 2.0 | 2.0 |
| Other crop seeds, ↑ | 10 / kg | 20 / kg |
| Other distinguishable varieties, ↑ | 10 / kg | 20 / kg |
| Total weed seeds, ↑ | 10 / kg | 20 / kg |
| * Objectionable weed seed (no.), ↑ | 2 / kg | 5 / kg |
| Seeds infected by paddy bunt (no.), ↑ | 0.10 % | 0.50 % |
| Germination (%), ↓ | 80 | 80 |
| Moisture (%), ↑ | 13.0 | 13.0 |
| Vapour proof containers (%), ↑ | 8.0 | 8.0 |

↓: minimum; ↑ maximum; * Wild rice

# VARIETAL MAINTENANCE IN WHEAT AND *KHARIF* PULSES

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In modern agriculture, seed is a vehicle to deliver almost all agriculture based technological innovations to farmers so that they can exploit the genetic potential of new varieties. Therefore, it is one of the most important inputs in agricultural production and most of the other inputs are dependent on it. Seed is the end product of variety development programme and is the first and basic input in crop production. Hence, regular supply of quality seed is very important to realise worth of a variety. The variety developed with great efforts will deteriorate/ contaminate if no variety maintenance scheme followed. The contaminating forces vary in importance and intensity with the crop (Parlevliet 2007). The forces are: (i) mechanical mixing ii) natural out-crossing iii) volunteer plants iv) residual heterozygosity. v) mutation vi) selective influence of disease.

Availability of quality seed in sufficient quantity can be assured with a systematic seed production programme i.e. generation system of seed production. It will take care of factors responsible for deterioration of a variety.

**Different Classes of Seed:**

The Indian generation system of seed multiplication consists of three classes of seed i) Breeder seed ii) Foundation seed and iii) Certified seed

**Breeder Seed:** Breeder seed is seed or vegetative propagating material directly controlled by originating or sponsoring plant breeder of the breeding programme or institution and/or seed whose production is personally supervised by a qualified plant breeder and which provides the source for the initial and recurring increase of foundation seed. Breeder seed shall be genetically so pure as to guarantee that in the subsequent generation *i.e.* certified foundation class shall conform to the prescribed standards of genetic purity. Breeder seed tag will be of golden yellow colour.

**Foundation Seed:** Foundation seed shall be progeny of breeder seed or be produced from foundation seed, which can be clearly traced to breeder seed. Certification tag shall be of white colour.

**Certified Seed:** Certified seed shall be progeny of foundation seed and its production shall be so handled to maintain specific genetic identity and purity as per standard for the crops. Certified seed may also be progeny of certified seed provided this reproduction does not exceed three generations beyond foundation seed stage-I. Certified seed produced from certified seed shall not be eligible for further seed increase under certification. Certification tag shall be of blue colour.

**Variety Maintenance**

Variety maintenance can be defined as 'perpetuation of small stock of parental material through repeated multiplication following a precise procedure' (Laverack, 1994). The precise procedure is basically *plant-row method* where selected (true to the type) plants are evaluated by performance of their progenies. This basic method has been modified suitably depending on the crop.

**Self-Pollinated Crops**:

- Plant-row method: Greengram, chickpea, lentil, fieldpea
- Ear-row method: Wheat, barley
- Panicle-row method: Rice
- Cluster-row method: Cowpea (Yadav et al., 2003)

**Cross/Open-pollinated Crops:**

- Reserve seed method: Pigeonpea, mustard
- Mass selection: Sorghum, mustard, pigeonpea

The variety maintenance/maintenance breeding programme helps in

a) Purification and maintenance of variety and consequent nucleus seed production

b) Reduction in amount of rogueing required in large breeder seed production plots

c) Removal of certain specific off types which can be detected only at nucleus seed production stage and

d) Extension of productive life of varieties.

**Variety maintenance and nucleus seed production in wheat**

Wheat is a self pollinated crop and most of the cultivars are pure lines. So nucleus seed is produced by ear to row method as it is not feasible to take out single plants because of it being a tillering crop.

1) Single ears heads (300-500) from plants typical of the variety are selected from nucleus seed production plot/ basic material supplied by the breeder.

2) These selected ears heads are threshed individually. The threshed seed is examined critically for colour, shape and size. The seed packets that are not true representative of the variety are rejected.

3) Individual ear seed is planted in a single row plot of 2.5m. The ear rows are examined critically at intervals during the entire growth period.

4) Variant rows or rows with deviant plant(s) and diseased & poor rows are discarded as and when detected.
5) Single ears are selected from the rows retained at the end for next maintenance cycle.
6) Rows retained at the end are harvested and threshed individually for sowing in NSS-II. If breeder seed requirement is very low (>25-30q), the individual ear-row produce may be bulked to constitute NSS-I. When breeder seed requirement is much higher, we should go for NSS-II. Production of nucleus seed stage-II will provide another chance to detect off-types.
7) In NSS-II, individual ear-row produce is planted in larger plots (usually having 8 rows of 8m) called Ear-Row Progeny plots by a plot drill.

8) The ear-row progeny plots are again critically examined at various growth stages. Variant plots are discarded. Plots having a few off-type plants are retained after roguing. For this, breeder/seed producer takes decision by his experience how many plants to be rogued out.
9) Plots retained at the end are harvested and threshed in bulk. This constitutes NSS-II and is further used for breeder seed production.

Wheat is a self pollinated crop but certain amount of out crossing may take place. Therefore, to avoid such out crossing; the variety maintenance/nucleus seed production plot should be surrounded by seed crop (preferably breeder seed crop) of the same variety.

Common wheat (Triticum aestivum L.) is a polyploid species of complex structure (2n=6x=42) which can tolerate well both chromosomal deficiency and duplication for each 21 chromosomes (Giura, 2009). That is why tall off-types are very frequent in wheat because of deletion/absence of height reducing genes present on B and D genomes. In addition to plant height, there may also be variation with respect to leaf attitude & width, pigmentation of auricle; leaf sheath, leaf blade, peduncle and ear waxiness; ear shape, size, density and colour; awns presence, attitude and colour etc.

**Variety maintenance and nucleus seed production in Kharif pulses**

Major pulse crops grown in *Kharif* season are greengran, blackgram, cowpea and pigeonpea. Except pigeonpea, all these crops are self pollinated; so plant-

row method as such or with little modification is used for variety maintenance. Pigeonpea is often cross pollinated; therefore reserve seed method, a modification of plant-row method, is used for its varietal maintenance.

**Variety maintenance in self pollinated pulses:**

Green gram is a self pollinated crop and variety maintenance is done by plant row method.

- Single plants (300-400) typical of the variety are selected, harvested and threshed individually.
- The single plant seed is screened critically in the lab for seed characters. Variant single plant seed or packet with one or more variant seed is rejected.
- Individual plant seed packets retained after screening are planted in single row plots of 5m each.
- Individual plant rows are critically examined at critical growth stages. Variant row or rows with one or more variant plant are rejected as and when detected.
- The remaining rows are harvested & threshed individually; and plant-row seed is again critically examined for seed characters. Variant seed packet or with variant seed(s) are rejected. The packets finally retained at the end are bulked to constitute nucleus seed.

If the nucleus seed requirement is large, we may go for NSS-II as in case of wheat, i.e. raising individual plant progeny in to a larger plot. The same methodology may be adopted in black gram. Cowpea plant has generally twining growth habit. So it may not be possible to take out single plants. Therefore, the plant row method has been modified to *cluster row method*, where single cluster is selected instead of single plant. A single cluster may have 2-4 pods depending upon variety. Rest of the method is similar to that followed in greengram

**Variety maintenance in pigeonpea**

Pigeonpea is a often cross pollinated crop where plant row method as such is not very effective as it does not exclude genetic contamination through pollen of variant/off type plants. So the method is modified to rest or reserve seed method.

1) Single plants typical of the variety are selected from central part of a seed crop plot
2) These selected single plants are harvested and threshed individually. The threshed seed is examined critically for colour, shape and size. The seed packets that are not true representative of the variety are rejected. The selected individual plant seed is divided into two parts maintaining their identity (serial number of the selected plant is written on both of the packets).


3) In the next season, one part of the seed from selected plants is sown in plant rows for evaluation. The remaining seed (other part) of each plant is stored. It is very important to maintain identity of the plant row and the reserve seed (e.g. for a particular plant seed, the row planted from one part of the seed and the reserve seed stored should be given the same number)


4) Individual plant progeny rows are examined critically throughout growing season. Any row with one or more off types is rejected as when detected.
5) At the time of flowering it should be examined daily or at alternate days to minimise genetic contamination from off type plants. All the rejected rows should be removed as and when detected.
6) Plant rows true to the variety for morphological characters are retained. These rows are harvested separately.
7) Seed from individual plant progeny rows is again examined for colour, shape, and size. The plant rows seed not conforming to the variety is rejected.
8) Reserved seed of single plants which produced true to type progenies is bulked to constitute the nucleus seed.
This bulked seed is planted in isolation for further multiplication or breeder seed production.
Since this procedure is laborious and difficult to practice, it is followed mainly for purification of varieties. For yearly maintenance, breeders select a large number of true to type plants from central part of the breeder seed plot and bulk seed of these selected plants to constitute nucleus seed. This method is also known as *mass selection*. This may be practised once the variety has been purified.

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# GOOD AGRICULTURAL PRACTICES FOR QUALITY SEED PRODUCTION

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Seed is the cheapest and most effective agricultural input. By being often the central input in the production package, quality seeds of improved varieties play an important role in modern agriculture. Raising of a seed crop differs from raising of a commercial crop in several respects. Special methods and precautions are needed to produce seed of desired quality. Important agronomical considerations in the production of high quality seed are discussed below:

## Seed certification standards

In India seed certification standards havebeen prescribed for foundation and certifiedseed.

There are two types of standards:

- ***Field standards*** which apply to standing crop viz. isolationrequirement, maximum permissible level of off types, inseparable other crop plants, objectionable weed plants, pollen shedders(in male-sterile or A lines), plants infectedby seed borne diseases etc.
- ***Seed standards*** which are applicable at seed level viz. genetic purity, physical purity, germination, other crop seeds, weed seeds, moisture content etc.

## Selection of an Agro-climatic region

A crop/variety to be grown for seed production in an area must be adapted to the photoperiod and temperature conditions prevailing in that area. Regions of moderate rainfall and humidity are more suited to seed production than regions of high rainfall and humidity. Excessive dew and rains hindrance in normal pollination causes lower seed setmost crops require dry sunny weather and moderate temperature during flowering. Too high temperature causes pollen desiccation and lower seed set. Hot dry weather prevailing during flowering in vegetables, legumes and fruit trees fails to set seed. In wind pollinated crops bright sunny weather with gentle winds cause an even flow of pollen over the crop which is conducive for best pollination and seed set. Very cold temperatures may also damage the seed quality during early maturation stages. Apart from affecting pollination excessive rainfall leads to higher incidence of diseases and insects and makes seed harvesting difficult. It may also result in delayed maturity pre germination in standing crops. Strong winds and heavy rainfall near harvest time causes seed losses due to shattering. Hence while selecting areas for seed production the all the above factors like sunshine,

moderate rainfall and the absence of strong winds have a decided advantage for production of quality seed.

**Selection of land**

Land to be used for seed production should be fertile, well drained and free of volunteer plants (self-sown plants). Volunteer plants are a serious problem in Brassica species. Self-sown plants continue to appear for 3 to 4 years. Volunteer plants is also a problem in legumes, pearl millet, sorghum and rice. However, when rice is transplanted after puddling the soil, volunteer plants are rare. There is no problem of volunteer plants in crops like maize and wheat. Fields heavily infested with objectionable weeds (Phalaris in wheat or Rumex Species in spinach)) should be avoided unless effective weed control measures are available.

Genetic contamination with unwanted pollen can be minimized by keeping in mind prevalent wind direction at flowering stage at a particular location. Fields to be selected for seed production should be chosen in such a way that they do not lie in the downwind direction side of the possible contaminant source.

| Crop | Frequency of volunteer plants |
|---|---|
| Rice | One year |
| Field pea | One year |
| Lentil | One year |
| Mustard | Three years |
| Lathyrus | One year |
| Berseem | Three year |

**Field standards:**

***Isolation:***

- The seed production plot must be isolatedfrom various sources of contamination by acertain minimum distance known as isolationdistance. Isolation is more important in cross-pollinatedcrops to avoid geneticcontamination through cross pollination bywind/insect borne pollen whereas in strictlyself-pollinated crops it is mainly to avoidmechanical mixture from adjoining plots.
- Sometimes distance isolation is not feasible time isolation can be given
- Time isolation is not permitted in tillering and branching crops (pearl millet, sorghum etc.)and crops with indeterminate flowering habit (Indian rape seed and mustard, pigeon peaetc.).
- Differential blooming dates (Time Isolation)are permitted if there is an interval ofabout 25-30 days between blooming of two varieties for crops like maize (Inbreds and Foundation Single Crosses), time isolation is effective.
- In cross pollinated crops keeping ofbeehives helps in pollination resulting inhigher yields. However, the number ofbeehives kept for pollination should

be optimum. More beehivesmay induce the bees to visit other pollensources, which leads to seed contamination.

**Green Manuring**

- Green manuring with comparatively low cost, is an effective way to improve the nutrient availability (nitrogen, phosphorus, potash and other micro nutrients) and soil fertility status.
- Leguminous crop like Dhaincha (*Sesbania aculeata*) is more advantageous as it does not require nitrogen but improves the soil nitrogen through biological nitrogen fixation.
- After harvest of wheat in the month of April- May the land is irrigated and dhaincha seeds are broadcasted @ 50 kg/ha in standing water. 50-60 days crop is ploughed back in the soil adding green matter ranging from 10-15 tones/ha.
- *Sesbania aculeata* can provide up to 60-80 kg N /ha equivalent to 3-4 tons of dry mass/ha. The burying of the whole crop is very much beneficial because all the nitrogen fixed by bacteria is returned back to the soil along with carbon for a longer time in organic form.
- The organic matter provides greater surface area for retaining all nutrients against leaching losses and provides increased water holding capacity.

**Method of sowing**

The seed crop should be sown in lines with a seed drill/planting equipment. One direction line sowing helps in cultural operations and inspection. The seed crop must be sown in such a may that it facilitates the movement of the

personnel to effectively execute rogueing. Skipping one row after every eight rows by plugging the central seed dropper pipe of a 9-tyne seed drill has been found to be very convenient and effective in case of wheat. Paired row planting in rice, green gram and chickpea is very useful. In crops like maize, pearl millet, sorghum, pigeon pea etc. the row to row spacing is wide enough for the purpose of rogueing.

**Time of sowing**

Time of sowing enormously affects the growth, yield and quality of seed crops. Time of sowing should match with the duration of the variety as well photoperiod and temperature requirements for flowering and seed maturation. In rice, delayed planting after 3rd week of July results in lower yield and quality due to low temperatures which coincides with flowering, seed filling and seed maturity stages. In case of forage sorghum and pearl millet, the sowing time is to be adjusted so that crop avoids the pollen wash due to rains. Moongbean sown during late August escapes the problem of yellow mosaic virus compared to July sown crop. In berseem, to maximize forage production the optimum sowing time is between 25th October to 5th November.


**Influence of Transplanting Dates on growing degree days, seed yield and quality of Paddy PB 1509**

Transplanting on 10th July resulted in significantly higher days to flowering, growing degree days (GDD),growth, yield attributes and seed yield compared to 30th July transplanting. There was reduction of 33.4 percent in seed yield at 30 July transplanting compared to 10th July transplanting. Higher values of 1000 seed weight, seedling length, seedling dry weight and vigour index was observed in 10th July transplanting compared to 30thJuly transplanting

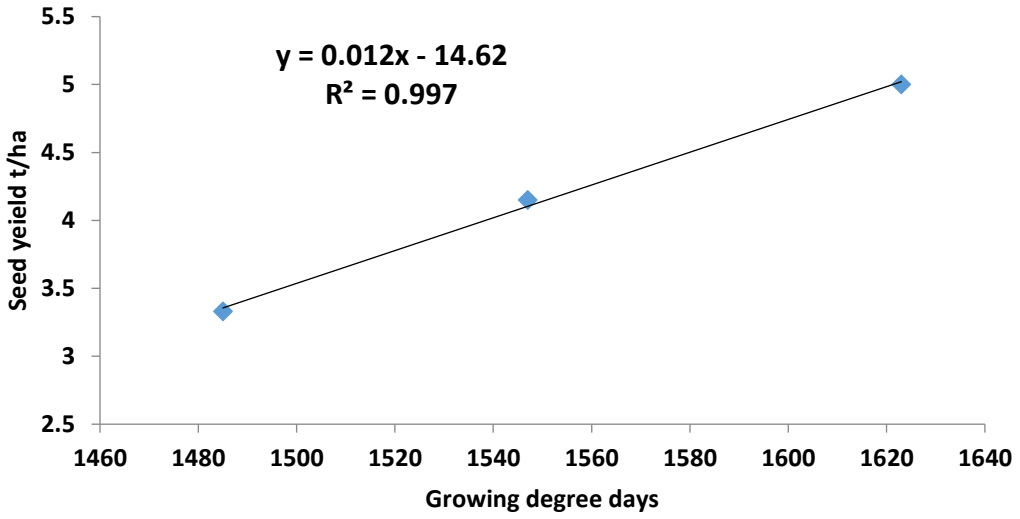


**Fig 1.Realtionship between GDD under different transplanting dates at 50 % flowering with seed yield**

**Effect of sowing dates on seed yield and quality of Berseem**

| Sowing dates | Green fodder yield(t/ha) | Seed yield kg/ha | Germination % |
|---|---|---|---|
| 25 October | 112.5 | 390.0 | 92.2 |
| 5 November | 108.7 | 400.0 | 91.5 |
| 15November | 91.0 | 320.0 | 91.8 |
| 25 November | 82.0 | 260.0 | 89.1 |
| CD at 5 % | 7.8 | 20.0 | NS |

There was reduction of 27.1 % in green fodder yield in 25th November sowing compared to 25th October sowing. Seed quality was not affected due to different sowing dates.

**Fertilizer Application**

In seed crops an adequate fertilization results in maximum yield of quality seed, better expression of plant type which facilitates roguing and thereby help in maintaining the genetic purity of crop as well. It will also influence concentration of nutrients in seeds, which may influence the seed germination. The adequate supply of nitrogen is beneficial, however excessive quantity of nitrogen can prolong the growing the period delay in maturity causing succulence increased risk of pests and diseases, this necessitates the estimation of optimum dose of nitrogen for quality seed production.

Timing of application of fertilizer is important. Excessive nitrogen during early stages will result in more vegetative growth, reduced fruiting and increased risk of lodging which reduces the seed yield and quality. Split application of

nitrogenous fertilizer is found to be advantageous. An adequate supply of both phosphorus and potassium improves the vigour and quality of crops.

Phosphorus encourages the formation of new cells, promotes root growth, hastens leaf development, emergence of ears, and formation of grains and hastens maturity. Phosphorus deficiency results in overall stunted growth, purplish discolouration of the stem and foliage owing to abnormal increase in sugar content and the formation of anthocyanin.

Potassium also plays an important role in flowering and seed development. An adequate supply improves the photosynthetic efficiency, synthesis of proteins and fat metabolism in oil crops. Potassium deficiency is usually accompanied by a general loss of dark green colour, weakening of straw, straw breakage in corn and sorghum and reduced crop yields. In the rice wheat rotation in the indo gangetic plains deficiency of zinc, manganese and boron are emerging which are required to be supplemented by chemicals.

**Weed Control in Seed crops**

**Source of Weed Infestation**

**i. Seed**

Planting un-clean (especially farmer-saved) seed which is often of lower quality and contain unknown quantities of weed seed may result in the introduction of weeds not previously observed on the farm. In India most of the peasants use their own saved seeds, which are full of weeds, so peasant cannot deny the entry and establishment of new weeds in an area not infected with it yet. During combine harvesting weeds seeds are also harvested along with crop. It is particularly true in case of winter grains. Current panic in India about *Parthinium hysterophorus* (Congress grass) and a wide spread occurrence of *Phlaris minor* (Wild canary grass) in winter crop through imported wheat grain in India is the best example of international spread of weed seeds.

**ii. Weed Seed Bank**

When any existing weed is permitted to mature and produce abundance seeds, these seeds enter in to soil seed bank and become source for future infestation. Seed rain is the new weed seeds which are periodically added to the existing seed population in soil by weeds growing *in situ.* Seed rain supplements the reservoir of weed seeds (nearly 500,000 per m2) (Das, TK 2013) in soil. Seed bank consists of:

- Surface germinating weed seeds – Surface to 2.5cm depth
- Shallow germinating weed seeds-2.5-5 cm depth
- Deep germinating weed seeds- 5-10 cm depth

**Table 1:** Seed Production of Selected weed species

| **Weed species** | **Seeds /plant** | **1000 seed wt (g)** | **Weed species** | **Seeds/ plant** | **1000 seed wt (g)** |
|---|---|---|---|---|---|
| Avena fatua | 250 | 17.52 | Echinichloa crusgalli | 7160 | 1.40 |
| Brassica nigra | 13400 | 1.70 | Rumex crispus | 29500 | 1.40 |
| Chenopodium | 72450 | 0.700 | Cyperus esculantus | 2420 | 0.19 |
| Cenchrus spp. | 1110 | 6.75 | Medicago lupilina | 2350 | 1.20 |
| Cirsium arvense | 680 | 1.57 | Phalaris minor | 10000-30000 | 2.0 |
| Cuscuta spp. | 16000 | 0.77 | Trianthema spp | 52000 | 1.5 |

**Objectionable weeds associated with seed crops**

Certain weed species have been designated as objectionable (whose seed separation is difficult once mixed with seed crop) as per Indian Minimum Seed Certification standards, maximum number of weed seeds permitted in foundation/ certified seeds for different crops are listed in table below

**Table 2:** Some objectionable weed species and their permissible limit for foundation and certified seeds of different crops

| **Crop** | **Objectionable weed** | **Permissible weed seed admixture limits** | | | |
|---|---|---|---|---|---|
| | | **Objectionable weeds (no/kg)** | | **Total weeds (No/kg)** | |
| | | **F S** | **CS** | **FS** | **CS** |
| Rice | Wild Rice or Red Rice (*Oryza sativa var fatua*) | 2 | 5 (0.01%) | 10 | 20 (0.10%) |
| Wheat | Field bind weed (*convolvulus arvensis)* and Little canary grass (*Phalaris minor)* | 2 | 5 (0.01%) | 10 | 20 (0.015) |
| Oats | Wild Oats (Avena Fatua/ ludoviciana) | 2 | 5 | 10 | 20 |
| Sorghum | *Sorghum halipense* | - | - | None | None |
| Lentil | *Vicia hirsute* | None | None | None | None |
| Peas | Wild Pea(*Lathyrus aphaca*) | | | - | - |
| Mustard | Mexican Poppy (Argemone maxicana) | 5 | 10 (0.01%) | 10 | 20 (0.5%) |

| | | | | | |
|---|---|---|---|---|---|
| Berseem | Chicory (*Chicorium intybus)* | 5 | 10 | 10 | 20 |
| Fenugreek | White sweet clover (*Melilotus alba*) | 2 | 5 | 10 | 20 |
| Cucurbit | Wild cucurbit (Cucurbit species) | - | - | - | - |
| Musk melon | Oriental pickling melon | | | | |
| Water melon | Wild water melon (*Citrullus colocynthis* L.) | None | None | None | None |
| Okra | Wild lady finger (*Abelmoschus* spp) | None | None | None | None |

**Preventive Weed Management**

**Use certified, Pure and clean seeds & seedlings**

- Seed should be certified and purchased from some authentic sources. In India more than 65% of peasants use their own saved seeds, which are full of weeds, so peasant cannot deny the entry and establishment of new weeds in an area not infected with it yet. . So seeds should be purchased from authenticated source or must be certified. Seeds purchased from local market should be avoided or must b
- Weed seeds, propagating materials harrows, ploughs, intercultural equ planting operations and get carri equipment should be cleaned before
- Measures should be adopted to pre weed seeds, rhizomes and other irrigation water. Preventive measure before irrigation should be adopted.

**Knowledge of critical period of weed competition**

The critical period of weed crop competition is between early growth during which weeds can grow without affecting crop yield and the point after which weed growth does not affect yield. Establishing the critical period of competition is essential to develop effective and economical weed control measures. Researchers have pin pointed the optimum critical period for different crops (Table 3).

**Table 3.** Critical period of crop weed competition in seed crops

| Name of crop | Critical Period of weed | Number of days weeds can be left in competition | Number of days after which weeds can be left to |
|---|---|---|---|

| | competition | after crop emergence | compete with the crop |
|---|---|---|---|
| Rice | 40-120DAS | 40DAS | 120DAS |
| Wheat(irrigated) | 30-45DAS | 30DAS | 45DAS |
| Tomato | 15-30DAT | 30DAT | 15DAT |
| Onion bulb | 20-60DAT | 20DAT | 60DAT |
| Black gram | 10-40DAS | 10DAS | 40DAS |
| Cumin | 20-40DAS | 20DAS | 40DAS |
| Pea | 15-60DAS | 15DAS | 60DAS |
| Cowpea | 15-45DAS | 15DAS | 45DAS |
| Pigeon pea | 30-6-DAS | 30DAS | 60DAS |
| Sunflower | 25-43 DAS | 25DAS | 43DAS |
| Chickpea | 40-60 DAS | 40 DAS | 60 DAS |
| Sorghum | 15-45 DAS | 15 DAS | 45 DAS |
| Maize | 15-30 DAS | 15 DAS | 30 DAS |

**Agronomical/ cultural methods**

Fryer (1983) stated "A *good crop is the best weed killer*" therefore the farmers should adopt a good crop husbandry, so that crop should get initial growth advantage. Row spacing affect the weed competition in crops. Optimum spacing for crop/variety may be worked out which may be close enough to give competition to weeds but does not invite intra species/intra specific competition of weeds with crop plants. Seed rate and germination percent of seeds will determine the crop density. Normally higher seed rate will result higher density and lower weed density but seed rate can be increased only up to the optimum level that intra crop competition between crop plants does not occur. Under weed free conditions increasing seed rates may reduce the yield, however under weedy conditions, weed competition may be reduced to some extent but cannot be compared with weed free plot yield with optimum seed rate.

**Crop rotation**: crop rotation is considered a sound approach to control weeds diseases and insects and has been an age-old practice in India for maintain soil health and sustainable crop production. Crop rotation has been found to be highly effective in controlling many weeds like Striges in sorghum, Orobanche ramose in Brassicas and Solonaceuos crops, Phalaris minor in wheat by taking berseem in rotation, alfalfa if replaced by cereals for two-to three years for the control of cuscuta.

**Soil Solarization for Nursery weed management in vegetables**

Soil solarization (solar heating of soil) as pre planting soil treatment to control soil borne pathogens and weeds, which involves mulching of the soil with clear plastic films so as to trap the solar heat in the surface soil. The resultant

temperature increases would be lethal to soil inhibiting pathogens nematodes and weeds. The common mulch for this purpose is transparent polythene. Soil solarization technique is simple and easy to use by farmers. However its immediate application appears to be more promising in nursery areas and in high value crops.

Plastic mulches have to be intact to reduce the losses through latent heat fluxes. Before covering the seedbeds with polythene sheet mulch of 100 mm, the land has to be irrigated and brought to a good tilth by two disking and planking. Spread Polythene sheet on soil surface with all edges buried in the soil to make it airtight. Duration of 4 to 6 weeks is adequate under most of the situation

In North India following weeds were found to be sensitive to soil solarization: *Trianthema monogyna* L, *Digeria arvensis* L, *Echinochloa colona* L, *Elusine indica* L, *Dactyloctenium aegyptium* L, *Phalaris minor* L, *Chenopodium album, Rumex dentatus L, Fumeria indica* L, *Avena ludoviciana* L

Soil Solarization is very effective (90% control) against parasitic weeds like Broom rape (*Orobanche* spp.).


d) **Weed Management in paddy nursery**

Following options should be followed for weed management for paddy nursery:

a. Pretilachlor S (30.7EC)[Sofit] 50 ml/300 $m^2$ area with surfactant as pre emergence at 3-5 DAS mixed with sand should be broadcasted in standing water and allow the water to seep slowly for broad spectrum weed management
b. Pyrazosulfuron 6 ml/300 $m^2$ area as pre emergence at 1-3 DAS mixed with sand should be broadcasted in standing water and allow the water to seep slowly for broad spectrum weed management

**New technique for raising robust and weed free paddy nursery on gunny bags**

Nursery beds of 0.75m width, 20m long, and 2.5" raised and 45 cm apart with channel are to be constructed. For raising nursery for one hectare area 20 beds of 20m will be required. Sides of the gunny bags are plugged with mud so that

seed remains on the gunny bag even if some extra water comes on the gunny bags.


On each nursery bed 8-10 kg grounded farm yard manure, 200 g NPK and 25g of zinc sulphate is uniformly spread and is covered by water soaked gunny bags. Sprouted seeds start greening within 3-4 days and requires protection form birds. The irrigation water will seep beneath the gunny bag and care is taken not to overflow the water on the gunny bag.



**Advantages:**

- Gunny bags serve as impediment and do not allow the germinating weed seeds to emerge, which is a major problem in conventional nursery where it is difficult to recognize between rice and weed seedling during initial stages and many a times weed seedlings are transplanted along with rice seedlings.
- There is no earth adhered to roots which avoids separation of single seedling saves time and labour which is essential for seed production point of view.
- Seedling can be uprooted gently from the gunny bags which does not cause any mechanical injury to roots and reduces the risk of incidence of bakanae disease.
- Transportation of the nursery is easy; gunny bags can be rolled and be taken to the field directly.


- Labour does not require uprooting the nursery in the wet nursery bed as done in traditional nursery; rather it can be done outside the field providing some comfort to the laborers.
- Raising of nursery for seed crop, genetic purity is of paramount important. The problem of volunteer plants is encountered successfully as jute bag layer does not allow the germinating seeds to emerge and volunteer plants germinating in between the vacant spaces are removed manually. This becomes very important in case of parental lines of rice hybrid where the cost of seed is high.

**Chemical weed control in seed crops**

**Paddy**

- Pretilachlor 1250 ml/ha and Pyrazosulfuron 200-250ml/ha with surfactant at 1-3 DAT mixed with sand should be broadcasted in standing water and allow the water to seep slowly for broad spectrum weed management.
- Bispyriback sodium [Nomini gold] 250 ml/ha area as post emergence at 15-25 DAT mixed with sand should be broadcasted in standing water and allow the water to seep slowly for sedges and grasses weed management.

**Wheat Grassy weeds**

- Preemergence application of pendimethalin @ 1.0lit/ha (30EC) may be sprayed after sowing controls *Phalaris minor* and other broadleaf weeds however does not control Avena spp.
- Clodinafop –proparygyl (60g/ha), Sulfosulfuron (25g/ha) and Fenoxaprop-p- ethyl (100g/ha) and Penoxaden(50g/ha) are recommended for the control of resistant biotypes of Phalaris minor.

**Broadleaf weeds**

- Metsulfuron methyl (Algrip) 4g/ha and Carfentrazone ethyl 50g/ha 30-35 days after sowing could be ideal replacement of 2-4D to control perennial weeds like *Cirsium arvense* and *Convolvulus arvensis* besides controlling

*Coronopus dydimus*, *Fumaria Parvi flora,Rumex dentatus, Lathyrus apache* and *Anagali sarvensis*.

**Weed management in rabi seed pulses**

Field pea (*Pisumsativum*) Chick pea (*Cicer arietnum* L.)/ Lentil (*Lens culinaris* L.)

- Pre planting incorporation of Fluchloralin @ 0.75-1.0 kg/has recommended for controlling of annual grasses and some broad leaved weeds provided sufficient moisture present in soil at the time of application.
- Pendimethalin @ 0.75 kg/ha pre emergence followed by a shallow weeding at 30-35 DAS
- Alachlor @ 1.0 – 1.5 kg/ha followed by a shallow weeding at 30-35 DAS.

**Weed control in vegetables crops**

**Tomato, Brinjal and Chiili**

- Soil solarization using polythene mulch is an effective approach for the control of weeds in tomato, Brinzal and chilli nurseries. Soil solarization is done for 4-6 weeks during hot summer months of May and June using thin plastic film (50 to 100 u) to control the weeds.
- Use of pre emergence herbicide Pendimethalin and Alachlor @ 1.0kg/ha followed by HW at 30-35 days is the best approach for the weed control in tomato and brinjal

**Onion**

- Onion seed crop is significantly reduced in weedy conditions and weed flora consist of both grasses and broadleaf weeds. Application of preemergence herbicides viz. Pendimethalin 1.0kg/ha, Alachlor 1.0kg/ha and Oxyflorofen (0.150kg/ha) followed by one to two hand weedings are the best weed management approaches for bulb and seed crop.

**Garden Pea**

- Garden pea seed crop is affected by complex weed flora. Application of Pendimethalin @ 1.0kg/ha followed by one HW at 30 days after sowing has been recommended treatment for effective weed control. However application of preemergence Pendimethalin followed by post emergence herbicide Imazethapyr @ 75g/ha proves to be better option for the control of complex weed flora.

**Fenugreek**

- Preapplication of Pendimethalin (Stomp) @ 0.75 to 1.0 lit/ha may be applied to control annual grasses and some broadleaf weeds. Moisture availability at time of spraying of Pendimethalin increases the efficacy.

**Okra**

- Alachlor @ 1.0-to 2.0 kg/ha as pre emergence herbicide followed by one HW 30-35days after sowing
- Pre emergence application of Pendimethalin (Stomp) @ 0.75 to 1.0 lit/ha followed by one HW 30-35days after sowing is required to control annual grasses and some broadleaf weeds.

**Carrot and Radish**

- In both direct seeded and transplanted crop Pre emergence application of Pendimethalin (Stomp) @ 0.75 to 1.0 lit/ha may be applied

**Managing *Cyperus rotundus* by application technique of Glyphosate in Cucurbit**

- Purple Nut Sedge (Motha)(*Cyperus rotundus*L.) is the most troublesome and obnoxious weed of spring summer season. As the temperature increases during first fortnight of March, spring summer crops viz., muskmelon, watermelon and bottle gourd etc. get heavily infested with *Cyperus rotundus*(300 to 500 shoots per $m^2$).The losses due to this weed range from 10 - 80 per cent depending upon the weed density and flora.

**Herbicide application technique**

In wider spaced crops like cucurbits, (at three to five leaf stage) individual plants are fully covered with PVC pots and then blanket spray of 1 % glyphosate (41 % SL) @ 600 lit / ha is done on weed foliage after 20 days of sowing.After the spraying, the pots must be allowed to dry to avoid any herbicide droplet adhering to outer surface before shifting to other place. Pots should not be stacked during shifting and must be carried individually.

**Effect of Glyphosate on weeds**After application on weeds, glyphosate is rapidly absorbed by foliage and is Trans located to roots and other underground storage organs in sufficient quantities to kill the entire plant. Visible effects occur within 3-5 days for most of the annual weeds. *Cyperus rotundus* and other broad-leafed weeds start showing degeneration (Yellowing) within 6 to 8 days. The early control of weeds resulted in faster growth of wines and higher fruit / seed yield. Least competition from weeds resulted in increased 1000 seed weight and high vigoured seeds. The PVC pots did not show any chemical effect of herbicide hence could be reused for years together.

**Irrigation**

Continuous optimum moisture status in soil during crop growth period is necessary for higher seed yield and moisture stress free period during reproductive period is necessary for getting good quality seeds.

Various crops respond differently to moisture tension at different stages of growth. When the vegetative growth is rapid plants react quickly to moisture

stress. The new leaves do not develop fully and a general reduction in succulence follows. When water is available again the rapid growth takes place but the small leaves produced under water stress seldom catch up with plants not subjected to water stress. Field uniformity is the first pre-requisite for better water management in rice. At the time of transplanting 1-2cm of water level is optimum which may be increased to 4-5cm within a week and may be extended up to 20-30days after transplanting .Moisture stress free period during reproductive period is necessary for getting good quality seeds.
In basmati rice keeping the standing water conditions for first 25-30 days after transplanting and then irrigating 3 days after disappearance of ponding water saves 20-25% irrigation water.

**Effect of irrigation schedule on number of irrigations and seed yield of aromatic rice**

| Irrigation Schedule | Total no. of irrigations | Seed yield (t/ha) | |
|---|---|---|---|
| **Water stagnation up to** | | **PB – 1** | **Pusa - 1121** |
| 15DAT* | 16 | 2.38 | 2.50 |
| 30DAT * | 17 | 2.43 | 2.83 |
| 45DAT * | 19 | 2.50 | 2.65 |
| 60DAT * | 21 | 2.62 | 2.14 |

***** Irrigation followed by 3 days after disappearance of irrigation water

In wheat depending upon availability of irrigation water stage of irrigations have been identified. In case of six irrigations water is applied at CRI, Tillering, Jointing, Flowering, Milk grain and dough stage where as if availability is of three irrigation then CRI,Jointing and Milk grain stages are the appropriate stage for the production of good quality seed.. In garden pea, Chickpea and lentils one irrigation with sprinklers is helpful in the absence of winter rains. Under north Indian conditions higher moisture availability due to winter rains causes excessive vegetative growth in pea, chickpea and lentil results in increased vegetative growth, delayed maturity and reduced yield.

**Harvesting and Threshing**

One of the important factors that affect the quality of seed and its subsequent performance is the maturity. In rice early harvesting may have green kernels and high moisture contents where as delayed harvesting results in shattering and alternate


wetting and drying of seeds may lead to suncraks that reduces the seed quality. Our experimental evidences show that to acquire good quality aromatic rice, the crop may be harvested 25-30days after 50% flowering.

Calibration of the threshers, combine harvesters etc. must be properly set up to thresh the seed without inflicting damage to the seed. The crop should be harvested at a proper moisture so as to minimize the damage to the seed. Our experimental evidences show that to acquire good quality aromatic rice, the crop may be harvested 25-30 days after 50% flowering.

**Post-harvest handling of seed**

In hybrid seed production, where two parents are involved, the male parent rows are harvested first and moved to a distant place. The whole field is then inspected and broken or lodged male parent plants are removed from the female parent rows.

The hybrid seed on the female parent rows is then harvested. All our efforts in rogueing and care during earlier stages may go waste if proper care is not taken during harvesting, threshing, etc. to avoid mechanical mixing.

The threshers, combine harvesters, trailers, threshing floors, processing machinery etc. should be thoroughly cleaned in between handling of different varieties.


Thorough Cleaning of Combine Harvester Blade and Combine harvesting of breeder/TL seed of wheat

For preserving the seed viability and vigour paddy crop should not be harvested more than 18%moisture contents. The threshed seeds should be sun dried on pucca floor. The threshing floor must be clean, dry and free from previous variety seed. After threshing seed should be winnowed to remove weed seeds,

partially and unfilled seeds. This will help in drying the seed lot. After winnowing seed must be dried to 13% moisture contents before bagging.

**Prevention of mechanical mixture during sowing**

- While raising rice nursery care is to be taken for maintain the distance between the two beds and separate channels for variety. Cleaning of the bunds with crop plants and collection of the rogued material outside the field helps in avoiding the mixing of the seed crops with off types. 2- 4 border rows between the two varieties of wheat and rice and before and after sowing one variety, the seed drill needs a thorough cleaning of the pipes, seed cups and box. All cracks and crevices in the seed box as well as around and under the collecting and distributing mechanism must be cleaned with a stiff wire.
- To ensure that physical mixture due to negligence at sowing time does not occur, it is desirable that phenotypically contrasting varieties are alternated. In such a situation any seed drill resultant rogues can be easily spotted in the field and rogued out.
- All care has to be taken to avoid physical mixing of seed during sowing, different crop stages, harvesting, threshing, transportation and drying. It has to be ensured that the seed drill/planter is free from the seeds of previous variety.
- In hybrid seed production male and female parents must be planted in the recommended row ratio along with border rows of the male parent. Also row direction is very important in wind pollinated crops and it should be kept nearly perpendicular to the prevalent wind direction at flowering time. Harvesting of male lines first from the hybrid seed plot of rice helps in maintain the genetic purity of the seed.

# SEED QUALITY ASSURANCE SYSTEMS – A GLOBAL PERSPECTIVE

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Use of high-quality seed is fundamental to the success of agriculture. Variety superiority and seed quality can have a major impact on potential crop yield under a range of production conditions. Thus, crop improvement through simple selection of superior lines from a population (as farmers often do), or by adopting conventional plant breeding methods or modern tools and techniques of biotechnology, is only the first step. However, supply of seeds with high planting value to the end users, that is the farmers, serves the ultimate goal. Seeds not only serve as the delivery system for improved genetics, but also for a range of performance enhancement and crop protection substances. Therefore, if the quality of the seed is not up to the mark, even a variety with high genetic potential fails to perform satisfactorily. Seed quality assurance, which essentially includes genetic and physical purity, germination and vigour and seed health, is thus, a crucial step in crop husbandry. Therefore, a reliable system of quality assurance is key to the maintenance of the planting value of seed.

Different countries follow different laws for the purpose of seed quality assurance. While in most of the European countries including UK, only certified seed may be marketed and only seed of registered varieties can be certified. The Federal Government is responsible for registration of varieties (DUS and VCU testing). After registration Federal State Governments are responsible for seed certification. Notification and testing of varieties takes place on the level of a variety in one member state of the EU, seed can be marketed in the entire EU. The of the Federal States. In India variety registration is voluntary and seed certification is not compulsory. Only labelling is compulsory for sale of seed. Both variety registration and seed certification are voluntary. Marketing of certified seed is restricted to those States where varieties are notified. In other countries like USA certification is not compulsory, but VCU for the purpose of variety listing is required for the seed in commerce. In India, in addition to the Seeds Act there is an indirect provision for seed quality assurance under the PPV&FR Act, 2001. As per Section 41(3) of the Act the purchaser of a labeled seed of protected variety claim compensation for under performance.

## Seed Quality

The essential criteria of seed quality for meeting the minimum requirements of cultivation are i) Variety Purity, ii) Physical Purity (Pure Seed Fraction), iii) Germination and iv) Moisture. In addition, Seed Health is also an essential

quality parameter, particularly in case of designated pathogens and pests. In recent times seed is also tested for vigour to ascertain its planting value, though it is still a voluntary assessment. Many seed producing organization process the seed through a series of quality enhancement techniques, which include seed treatment with pesticides, biological, coatings with chemically active or inert substances, growth enhancers etc. Seed enhancement technologies are gaining increasing attention for their potential to confer greater disease resistance in seeds and improve seed vigor germinate and emerge well under a wide range of conditions.

Most countries in the world follow an internationally accepted system of seed quality testing, based on the procedures standardized and recommended by the International Seed Testing Association (ISTA), while adopting a quality assurance / quality control mechanism, as stipulated by their respective legislations. This is to ensure that the seed in the commercial supply chain meet the minimum standards (as prescribed by the designated authority) for different quality parameters. Given below are the basic principles, procedures and types of implementing systems adopted globally.

**Principle**

The basic principle of quality assurance requires that each seed container in the commercial use must be properly labeled, indicating the kind and the variety of seed, as well as the identity of the seed producing agency. It is also expected that the seed thus labeled meets the minimum standards of quality parameters. This is the first step of seed quality assurance by indicating that using the seed of a particular crop and variety will result in successful emergence and crop establishment. If the seed is of Certified Class, the variety identity and genetic purity is validated through examination of the source and other necessary information, field inspections etc. by an officially designated agency, either under the control of the Federal (Central) or the State governments or accredited by them. In addition, the processed seed is sampled and tested in authorized laboratories for other essential quality parameters. However, in case of uncertified or Labelled seed (also sometimes known as Quality Declared Seed), the variety purity as well as the quality standards of the seed are and declared (assured) by the seed producing agency itself. *Thus, any seed in commerce, whether certified or not, is expected to have been produced and processed in such manner, following such practices that ensure the user that the seed is of the genotype indicated and it meets the prescribed minimum quality standards.* In case of compulsory variety registration, the purchaser is also assured of the value for cultivation and use of a variety under a given set of agronomic conditions. However, in India variety registration and hence, VCU is not compulsory. A variety, whose performance is indicated by the company, based o its own field evaluation is permitted for sale.

**Testing Procedures**

As mentioned above most countries follow ISTA Rules and Procedures for seed quality analysis, with suitable modifications, wherever necessary. These are adopted as national seed testing protocols. Indian Seed Testing procedures are in principle those recommended by ISTA, with minor modifications brought in from time to time considering the experiences of the Seed Certification Agencies as well as on the request of the industry (eg. size of the submitted samples in case of vegetable hybrid seeds). All procedures are vetted by a Technical Sub-committee under DAC, Ministry of A&FW. In the US such procedures are vetted by a joint professional body comprising the Association of Official Seed Analysts (AOSA) and Society of Commercial Seed Analysts (SCSA).

It may be noted that since the development of testing procedures depend totally on the understanding of the underlying processes, each aspect requires in-depth research and consultations by the scientists. Hence, most countries rely on the scientific knowledge and expertise of the seed scientists, through various scientific and professional societies such as ISTA and AOSA/ SCSA, in developing and standardizing seed testing protocols. The periodicals published and workshops organized by these professional bodies play an important role in initiating new technologies and modifying and fine-tuning the existing procedures based on the scientific advancements. In India, while there is some representation of seed technologists in the Technical Committees, their involvement and participation need to be enhanced. Similarly, involvement of the professional society the Indian Society of Seed Technology can be thought of.

**Implementation**

In India, variety registration (Notification) and seed certification are not compulsory by law, whereas, labeling is mandatory. It is the similar system in many Asian countries. In recent years most states in India are enforcing avariety registration based on at least one year's field trial conducted on behalf of the Department of Agriculture/ Horticulture, by the State Agriculture Universities or other designated agencies. However, in a number of European countries like Germany, UK, all seed in the market must be of registered variety and certified. In India, as per the Section 8 of the Seeds Act, 1966, seed certification is undertaken by the designated Seed Certification Agencies (SCA) under the State Departments of Agriculture or Autonomous, for the seeds produced within their areas of jurisdiction. There are 24 SCAs in the country, 10 of these are also Designated Authorities for OECD certification. Similarly, in the U.S., the seed certification system and the authority for seed certification is governed by the Federal and state law. The recognized official seed certifying agencies are the members of the Association of Official Seed Certifying Agencies (AOSCA) which establishes minimum seed certification standards are used

throughout the United States/ N Americas. Many of these Agencies can certify seed for OECD.

Certified seed production can be undertaken by any individual or organization in India. State Seeds Corporations, National Seeds Corporation, State Departments of Agriculture, SAUs, ICAR institutes, Private Companies, Cooperatives and individual farmers are producing certified seed. There are six basic steps for seed certification. These are:

- Scrutiny of application
- Verification of seed source and class
- Field inspections to verify conformity to the land requirement and prescribed field standards
- Supervision at post-harvest stages including processing and packaging
- Seed sampling and analysis in a notified lab to verify conformity to the prescribed standards
- Grant of certificate and certification tags, tagging and sealing.

In case of the Labeled Seed, the producing agency is responsible for ascertaining the variety identity and purity, whereas the processed seed may be tested on their own, or be sent to a notified seed-testing laboratory for quality analysis.

In India, following information are to be provided for labeling:

Name and Address of the Producing agency
Name of the kind and variety
Net weight
Purity (Pure seed fraction)
Germination
Moisture content
Date of testing

It must be clearly mentioned if the seed is treated with any pesticide and safety instructions.

On the other hand a typical seed label in the US contains the following information:

Name and Complete Address of Seed Company
Name of the Variety / Kind
Net weight
Lot No.
Pure seed
Other crop seed
Inert matter
Weed seed
Germination
Hard seed
Date tested

It must be clearly mentioned if the seed is treated with any pesticide and safety instructions.

In most countries with a seed legislation in place, including India (under Seed Control Order, 1983), any commercial seed packet / container can be sampled at any time by the authorized agency and tested in notified laboratory for its quality standards. Similarly, the purchaser can also file a complaint against the producing and / or selling agency, if the seed is found to be inferior in quality. Action can be taken including fine/ imprisonment and other punitive measures, against the seed producing and or selling agencies if the seed is found to be sub-standard. Thus, the purpose of random market check is to deter anyone to sell/ intend to sell a seed, which does not ensure the minimum standards of purity, germination and other essential parameters. Most seed companies, therefore take all such measures which will ensure high seed quality during production, processing, storage and sale. In addition to testing seeds as per ISTA recommended procedures, many seed producing companies pass their produce through several additional tests, viz., molecular / protein marker test for variety identity and purity, immunological tests for identification of seed borne/ transmitted pathogens. Every responsible seed company wants to build and maintain the reputation and goodwill of the company by offering high quality seeds in the market. This helps in capturing the market by winning farmers' trust. In case of GM crops, every seed lot also needs to be tested for trait purity. In India, the designated laboratory for Bt cotton is CICR, Nagpur.

The purity of any seed begins with the genetic purity, hence the seed producers and Certifying agency must assure growers that the seed they buy is accurately labeled with the correct crop and variety. Seed purity also indicates the actual percentage of Pure seeds and level of contamination by seeds of other varieties or species, including that of weed seeds if any. The physical purity of seed, which also refers to the percentage of other inert materials such as dirt or plant residues and germination of the seed lot helps determine the buyer the exact quantity of Pure Live Seed and can adjust the quantity required to achieve the desired plant population. Hence these must be indicated on the label. In addition, seeds may also need to be tested for the presence of seed-borne diseases and indicated so.

To maintain genetic purity producers take many steps such as verifying the source and class of seed, planting seeds on clean land which has not been used to grow the same crop in the recent past, removing all unwanted plants, such as rogues, plants of other varieties, diseased plants etc. at the right stage; maintaining physical isolation – distance isolation, time isolation or growing seed crop inside protection , use emasculation and pollination – to ensure that pollination occurs only between desired plant varieties, harvesting and handling in such manners as to avoid all possible chances of physical contamination.

Similarly, sampling, testing, labeling and storage requires the adoption of best practices to ensure that the quality of the seed is assessed accurately and maintained well till its use. In many western countries self certification by the seed producing company is permitted by law, provided the organization and its laboratory meets quality assurance standards and is accredited by the designated authority for the same. Some countries (EU and Americas) also allow seed inspection, sampling and testing by the independent licensed Seed inspectors and Seed Analysts whose proficiency is regularly monitored by the authorized agencies and upgraded if required.

# PRINCIPLES AND PROCEDURES OF SEED SAMPLING

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Indian seed industry working on its three basic phases and seed act 1966 regulation of sale of seeds of notified kinds or varieties and provide the safe guard assurance to the farmers for seed quality. The Act came into force throughout the country including Jammu and Kashmir on 2nd October 1969. Seed was declared as an essential commodity under the Essential Commodities Act (1955). For the quality assurance all three phases of seed business control by the government regulation in the seed production, seed processing and seed marketing. Different activities of seed production at different phases like seed production in the field, seed processing, seed storage and seed marketing, the seed quality insured by the seed certification agencies and department of agriculture in the state. During seed marketing seed dealer to selling process controlled by state department of agriculture. After harvesting of seed plots different seed lots should formed and seed transported for seed processing & seed quality up-gradation purpose. Seed quality assurance to the farmers it is necessary to take the representative samples from all representative quantity of seed produced. Therefore, seed sampling and sampling procedure should be followed for seed quality assessment of seed produced for seed labeling purpose in the market.

Every year India invests huge money on import of quality seed of agricultural-horticultural crops in the country.

India import of quality seed of agricultural-horticultural crops in the country.

| Year | Vegetable Crops (Mt) | Flower seed (Mt) | Field crops (Mt) | Total (Mt) | Vegetable crops (USD Million) | Flower seed (USD Million) | Field crops (USD Million) | Total (USD Million) |
|---|---|---|---|---|---|---|---|---|
| **2015** | 3385 | 8 | 27903 | **31296** | 73 | 7 | 35 | **115** |
| **2014** | 2340 | 5 | 25643 | **27988** | 67 | 6 | 32 | **105** |

Source: ISF compilation based on official statistics and international seed trade reports

The purpose and objective of seed samples are to seed quality evaluation, technical purity, and other seeds by number, germination, viability, seed moisture testing, variety testing, GMO testing, field plots and seed health testing.

For 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 component 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. Therefore knowledge of seed lot formation will known to all working manpower in the seed laboratory.

**1) PROCEDURE FOR CONSTRUCTION OF LOT NUMBERS:** The lot number will have four parts. Each part will signify and conform to the details given as under:

**a) First Part** This shall be called the "Month-Year Code" and will indicate the month and year in which the concerned seed crop was harvested. The month will be represented by its abbreviated form and the year will be represented by the last two digits of the calendar year, such as 89 for 1989 A.D., 90 for 1990 A.D., 00 for 2000 A.D. and 01 for 2001 A.D. The abbreviated form to be used for each month is given as under:

***Month Abbreviated form*** January JAN February FEB March MAR April APR May MAY June JUN July JUL August AUG September SEP October OCT November NOV December DEC

**b) Second Part** This shall be called the "Production Location Code" and will indicate the State or Union Territory, where the concerned seed field(s) was/were located. For this purpose, each State and Union Territory is allotted a permanent numerical as shown below:

| Code | State or Union Territory | Code | State or Union Territory | Code | State or Union Territory |
|---|---|---|---|---|---|
| 01 | Andhra Pradesh | 13 | Maharashtra | 25 | West Bengal |
| 02 | Arunachal Pradesh | 14 | Manipur | 26 | Andaman & Nicobar Islands |
| 03 | Assam | 15 | Meghalaya | 27 | Chandigarh |
| 04 | Bihar | 16 | Mizoram | 28 | Dadra & Nagar Haveli |
| 05 | Goa | 17 | Nagaland | 29 | Delhi |
| 06 | Gujarat | 18 | Odisha | 30 | Daman & Diu |
| 07 | Haryana | 19 | Punjab | 31 | Lakshadweep |
| 08 | Himachal Pradesh | 20 | Rajasthan | 32 | Puducherry |
| 09 | Jammu & Kashmir | 21 | Sikkim | 33 | Uttarakhand |
| 10 | Karnataka | 22 | Tamil Nadu | 34 | Chhattisgarh |
| 11 | Kerala | 23 | Tripura | 35 | Jharkhand |
| 12 | Madhya Pradesh | 24 | Uttar Pradesh | | |

**c) Third Part**

(a) This shall be called the "Processing Plant Code" and will indicate the seed processing plant where the relevant lot was processed. For this purpose, the Certification Agency shall allot a numerical commencing from 01 to each seed processing plant within its jurisdiction irrespective to whom it belongs.

(b) In crops like groundnut, potato, sweet potato, tapioca etc. which may not be brought to the processing plant for post-harvest operations including grading, sorting, packing etc. the individual centre where such operations are carried out will be treated as a "Processing Plant" for assigning the Processing Plant Code.

**d) Fourth Part** This shall be called the "Seed Produce Code". It will indicate ultimate serial number of an individual lot. The procedure for assigning this code will be based on unit of certification. For this purpose, the Certification Agency shall allot a numerical commencing from 01 to each unit of certification. However, if seeds of more than one unit are bulked together then bulked unit will be treated as one unit. If the quantity of the seed from one unit of certification exceeds the maximum limit of lot size, it will be further sub-divided into a separate lot, in such cases Roman numerical commencing from (i) will be suffixed with seed produce code within brackets, for example if 586 quintals wheat seed is obtained from one unit of certification, it will necessitate the sub-division of the produce into three separate lots (200, 200 and 186 qtls.) if 01 is allotted to "Seed Produce Code", then sub-divided lots will be represented as 01 (i), 01(ii) and 01(iii). 5. All the four parts of the lot number shall be written in series with a 'dash (-)' between first, second, third and fourth parts to distinctly indicate the code number of each part. An example is shown below: Lot No. --- MAY 88-12-01-01, MAY 88 --- Seed harvested in May 1988. 12 --- Seed crop raised in Madhya Pradesh 01 --- Seed processed in a processing plant identified as number 01 by the Madhya Pradesh State Seed Certification Agency. 01 --- Seed Produce Code which will trace to the particular unit of certification.

**Crop, Size of seed lot (Kg), Size of submitted sample (g), Size of working, Sample for purity analysis (g) and Sample count of other species (g)**

| Crop | Size of seed lot (Kg) | Size of submitted sample (g) | Size of working Sample for purity analysis (g) | Sample count of other species (g) |
|---|---|---|---|---|
| Paddy | 20,000 | 400 | 40 | 400 |
| Wheat | 20,000 | 1000 | 120 | 1000 |
| Maize | 40,000 | 1000 | 900 | 1000 |
| Sorghum | 10,000 | 900 | 90 | 900 |
| Bajra | 10,000 | 150 | 15 | 150 |
| Redgram | 20,000 | 1000 | 300 | 1000 |
| Greengram | 20,000 | 1000 | 120 | 1000 |
| Blackgram | 20,000 | 1000 | 150 | 1000 |

| | | | | |
|---|---|---|---|---|
| Bengalgram | 20,000 | 1000 | 1000 | 1000 |
| Cowpea | 20,000 | 1000 | 400 | 1000 |
| Soybean | 20,000 | 1000 | 500 | 1000 |
| Groundnut(pods) | 20,000 | 1000 | 1000 | 1000 |
| Groundnut(Kernels) | 20,000 | 1000 | 600 | 1000 |
| Gingelly | 10,000 | 70 | 7 | 70 |
| Sunflower(variety) | 20,000 | 1000 | 250 | 1000 |
| Sunflower(Hybrid) | 20,000 | 1000 | 125 | 250 |
| Cotton linted (variety) | 20,000 | 1000 | 350 | 1000 |
| Cotton delinted (variety) | 20,000 | 350 | 35 | 350 |
| Cotton linted (hybrid) | 20,000 | 350 | 35 | 350 |
| Cotton delinted (hybrid) | 20,000 | 250 | 25 | 250 |
| Brinjal | 10,000 | 150 | 15 | 150 |
| Chillies | 10,000 | 150 | 15 | 150 |
| Bhendi | 10,000 | 150 | 15 | 150 |
| Tomato(variety) | 10,000 | 70 | 7 | 70 |
| Tomato(hybrid) | 10,000 | 7 | 7 | 7 |
| Cabbage | 10,000 | 100 | 10 | 100 |
| Cauliflower | 10,000 | 100 | 10 | 100 |
| Knolkhol | 10,000 | 100 | 10 | 100 |

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

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

ii) **Seed lot:** It is specified quantity of seed, physically identifiable, in respect of which a seed test certificate can be issued.

iii) **Primary sample:** Small portion taken from one point in the lot.

iv) **Composite sample:** Is formed by combining and mixing all primary samples taken from the lot.

v) **Submitted sample:** Sample submitted to seed testing laboratory .The size of the submitted sample is specified in the Seed Testing Rules.

vi) **Working Sample:** Is a sub sample taken from Submitted Sample in the laboratory, on which one of the quality test is made.

**General Principles of Sampling:** Under seed law enforcement programme only trained and experienced officials are authorize to undertake sampling and he has to:

Give notice to such intention to the person from whom he intends to take sample.

Take three representative samples in the prescribed manner and mark & seal.

One sample to be deliver to the person from whom it has been taken

Second to be sent for analysis to the Seed Analyst of the area.

Third to be retained for any legal proceedings.

At least two persons should be present and obtain the signature of both the witnesses on from VII 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 then sampler should check the following information on the seed certification tag:

i. Name & Address of certification agency ii. Kind & Variety iii. Lot No. iv. Name & address of certified seed producer v. Date of issue of the certificate & its validity vi. Class & Designation of seed vii. Period during which the seed shall be used for sowing

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

Minimum sampling intensity for seed lots in containers : Sampling seed lots in containers between 15 and 100 kg

| **Containers in the seed lot** | **Minimum number of primary samples** |
|---|---|
| 1-4 | 3 samples from each container |
| 5-8 | 2 samples from each container |
| 9-15 | 1 samples from each container |
| 16-30 | 15 samples in total |

| | |
|---|---|
| 31-59 | 20 samples in total |
| 60 or more | 30 samples in total |

**Sampling seed lots in containers smaller than 15 kg**

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

**b. For seeds in bulk**

Up to - 500 kg : At least 5 Primary samples

501 - 3000 Kg : One primary sample for each 300 kg but not less than 5 primary samples

3001-20,000 Kg : One primary sample for each 500 kg but not less than 10 primary samples

20,001 and above : One primary sample for each 700 kg but not less than 40 primary samples

**INSTRUMENTS FOR TAKING SAMPLES FROM SEED LOTS**

**By hand**

a) Push the open hand in to the container
b) Close the hand with seeds inside
c) Withdraw the hand by taking great care that fingers remain tightly closed around the seeds so none may escape
d) In case of treated seeds, use appropriate gloves

**Principles of Hand Sampling**

This method can be used for all species but is most suitable for chaffy, non free-flowing grasses where the seed would be damaged by use of a trier, specialty seed (e.g., seed mats, seed tapes), retained samples stored in containers making sampling with use of a trier or divider not practical or where there could be separation and selection of the seed or where there could be separation and selection of the seed.

Examples of chaffy, non-free-flowing grasses include *Agropyron*, *Psathyrostachys* and *Elytrigia* (wheatgrasses), *Agrostis* (bentgrass and redtop), *Alopercurus*, *Bromus* (bromegrass), *Dactylis* (orchardgrass), *Elymus* (wildrye), *Festuca* (fescues),*Lolium* (ryegrasses), *Poa* (bluegrass), Native Species such as *Anthoxanthum*, *Arrhenatherrum*, *Axonopus*, *Chloris*, *Cynodon*, *Cynosurus*, *Deschampsia*, *Digitaria*, *Holcus*, *Melinis*, *Panicum*, *Paspalum*, *Pseudoroegneria*, *Trisetum* and *Zoysia*. All positions inside the seed container must be accessible. When it may be impossible to obtain samples from the lower parts of bags or bins, the seed sampler must request that the containers be partially or completely emptied in order to gain access to all positions of the container. The sampler must be able to reach the bottom of the container.

The following procedures must be followed when:

a. open containers are greater than 40 cm in depth; primary samples are taken directly from the open bag which has been emptied sufficiently to allow sampling from all parts of the bag. After sampling, the seed is repackaged into the appropriate container and sealed. This shall be done under the supervision of the sampler when the purpose of sampling is for the issuance of Orange International Seed Lot Certificates or for sampling under the AEP;
b. containers are less than 40 cm in depth; seed presented in this size container permits access to all parts of the seed lot containers. Primary samples are drawn. After sampling, the seed is repackaged into the appropriate container and sealed;
c. samples are taken from the seed stream as the container is emptied and refilled into a new container.
d. In the case of seed tapes or mats, the container(s) for sampling has to be opened and a sufficient number of units (tapes or mats) or a sufficient part, in the case of a long tape, has to be taken from the container.

**Procedure for Hand Sampling**

The following method must be used for hand sampling:

a. if the sampler is wearing long sleeved apparel, s/he must first verify that the sleeves are sufficiently rolled up so as not to capture seed or otherwise interfere with the sampling process;
b. the open hand is inserted through the top of the bag with fingers held tightly together, until the desired depth is reached;
c. the hand is then closed with the fingers held tightly together so that few, if any, seeds escape, and the hand slowly withdrawn;
d. this process is repeated a number of times in different parts of the seed lot and at different depths, until the required sample size is obtained.

**ii) Sampling with Tries or Samplers:**

The equipment listed in ISTA appendix I must be used for drawing official samples including export samples. This appendix lists the triers and sizes that are approved for the crop kind being sampled. When selecting the appropriate trier for sampling:

a. the trier must not select or separate seed during sampling, due to seed size, shape density, chaffiness or any other quality trait;
b. it must not damage the seed being sampled.

Each primary sample is obtained by passing the sampling equipment through the seed once. Each trier or pass of the sampling equipment is one primary sample.

**Triers:** When selecting the appropriate trier for sampling, the sampler should consider the species being sampled, the size and type of the containers. When sampling containers, all positions inside the container must be accessible. Appendix I specifies appropriate trier sizes for each crop kind. The principle on

which the trier size is based is that the width of the opening should be not less than two times the diameter of the seed (diameter meaning the longest part of the seed) for sampling. At no time is it permissible to use a trier that is smaller than that specified in Appendix I for the species being sampled unless the sampler provides evidence to the laboratory that the width of the opening is, at a minimum, two times the diameter of the seed. There are triers with single or multiple chambers and also combination types. These instruments may have more than one opening (slot/hole). The sampler must check the trier and other equipment for cleanliness before use. The sampler must state the type and size of trier or the method used for sampling on the Sample Submission Form (SSF) Appendix V. When the trier used is smaller than that specified in Appendix I for the species being sampled, the sampler must provide evidence that the width of the opening is, at a minimum, two times the diameter of the seed on the SSF.

***Nobbe Trier:*** This trier is a pointed tube with an oval opening near the pointed end. This trier is relativity compact and small, making it easy to transport. The risk of contamination is low as the trier is easy to keep clean. If sampling from the end of the bag, the trier must be long enough that the opening (slot/hole) reaches the centre of the bag. If sampling from the side of the bag, the trier must reach the opposite side. A Nobbe trier is suitable for sampling free-flowing seed in bags (legumes, timothy, rapeseed, mustard) but not in bulk. It may only be used horizontally. Its use is limited to penetrable containers.

**Procedure for Nobbe Trier Use**

The sampler must use the following procedure when sampling with a Nobbe trier:

a. the trier is inserted gently into the centre of the bag with the trier opening facing downwards;
b. the trier is inserted into the bag upwards at an angle of approximately 30 degrees to the horizontal:
    i. when sampling from the end of a container, the opening of the trier must reach the centre of the container. The trier is inserted as close to the bottom edge of the container as possible (i.e., below the stitching that seals the end of the bag to achieve the 30-degree angle);
    ii. when sampling from the side, the opening of the trier must reach the opposite side of the container. The trier is inserted at the bottom edge of the container such that the 30-degree angle is achieved;
c. the trier is then rotated through 180 degrees, bringing the hole to face upwards;
d. the trier is withdrawn:

i. when sampling from the end, with decreasing speed so that the quantity of seed obtained from successive locations increases progressively from the centre to the side of the container;
ii. when sampling from the side, with a constant speed;
iii. the trier should be gently agitated as it is being withdrawn to help maintain an even flow of seed. The trier must not be agitated without withdrawing it;

e. each primary sample must be placed into a suitable clean container(s) (pan, pails) to allow for checking for uniformity prior to adding to the previously drawn primary samples.

**Double Sleeve Trier**

This type of trier is suitable for sampling seed in bags, large containers or in bulk as is the case of sampling containers that are 100 kg or greater such as, mini-bulk containers or in static bulk lots of both small or large seeded crop kinds. This trier may be more suitable for drawing samples from the bottom rows of palletized containers that are stored at floor height than a Nobbe trier. This trier consists of a hollow tube with a close fitting inner tube such that seed cannot slip between them. A handle is fitted to the inner tube so that it can be twisted inside the outer tube. The outer tube has a solid pointed end. Openings (slots/holes) are cut into both the inner and outer tubes. The trier consists of:

a. multi-openings without partitions; or
b. multi-openings with partitions.

The opening in the inner tube can be opened and closed by turning the inner tube until the openings in the inner and outer tubes are aligned. There is a greater risk of contamination with this type of trier. Care must be taken to verify that all the openings in both the inner and outer tubes are clean.

A double sleeve trier with partitions may be used:

a. horizontally;
b. vertically in both open and closed penetrable containers.

The contents of the entire trier represent one primary sample. A double sleeve trier without partitions may be used horizontally only. Care must be taken when closing the openings, as there is a risk of damaging the seed trapped between the edges of the slots. The risk of damaging the seed can be reduced by slowly closing the openings to the point when resistance is felt. There is no possibility of varying the amount of seed obtained from the inner and outer part of the container by adjusting the speed with which the trier is withdrawn as the trier draws the same size sample in each sampling action. The trier must always be long enough to reach the opposite end of the container on the diagonal. When sampling the containers vertically, the trier must be partitioned and reach the bottom of the container.

**Procedure for Multi-Opening Types Without Partitions**

This type of trier can only be used horizontally. The following method must be used:

a. the trier is carefully inserted diagonally on the horizontal plane into the container in the closed position until it reaches the opposite corner of the container. The outer tube opening must be facing upward. Care should be taken not to push the trier through the opposite corner of the container;
b. the trier is opened until the inner and outer openings are aligned and agitated slightly to allow the openings to fill;
c. the trier is gently closed (to point of resistance) and withdrawn;
d. each primary sample must be placed into a suitable clean container(s) (pan/pail) to allow for checking for uniformity.

**Procedure for Multi-Opening Types With Partitions**

This type of trier may be used horizontally or vertically. If the sampling is done on the horizontal plane, the method for unpartitioned triers is to be used. If the trier is to be used vertically:

a. the trier is carefully inserted into the top of the container in the closed position on an angle until it reaches the bottom of the container;
b. the trier is opened until the inner and outer openings align and agitated slightly to allow the openings to fill;
c. the trier is gently closed and withdrawn;
d. the primary sample is placed onto a clean long piece of paper or into a suitable clean container that is the same length as the trier to allow for checking for uniformity.

**Principles of Seed Stream Sampling:**

When the seed is processed in a closed system and is packed into sealed, marked containers, the primary samples may be taken from the seed stream. Sampling from the seed stream can be conducted manually or by seed sampling devices. When devices are controlled automatically, they are called automatic seed samplers. Where there are open flow streams, manual sampling from the stream at the end of processing may be the best technique. The advantage to this technique is that each individual primary sample can be examined for uniformity. For drawing stream samples, these conditions must be met:

a. the primary samples are to be taken during the process as the last step before the seed enters the containers which are to be sealed;
b. the equipment used for sampling must not select or separate seed during sampling, due to seed size, buoyance and chaffiness;
c. the entire cross section of the seed stream must be sampled;
d. seed entering the sampling instrument must not bounce out again;
e. sampling the flow of the seed stream should be at regular intervals, to the extent practical.

**Automatic Sampling**

There are many types and designs of automatic sampling devices. An automatic sampling device is to be used and maintained within a seed establishment's quality system. When an automatic sampling device is used for sampling for the purpose of:

a. the issuance of Orange International Seed Lot Certificates or for sampling under the automated entry processing (AEP), Canadian Food Inspection Agency (CFIA) must review and approve the validation system.
b. drawing an officially recognized sample by an establishment, the licensed sampler must validate the system and keep records.

The inspector may read the more details of automatic sampling. This approved process is to be used for automatic samplers that are used to obtain samples for the issuance of an Orange International Seed Lot Certificate by Seed Science and Technology Section (SSTS) or by a laboratory participating in the automated entry processing (AEP).

**Process:** If the automatic sampling device will be used for sampling for the issuance of Orange International Seed Lot Certificates, the company or applicant must send an application for accreditation to the Seed Science and Technology Section (SSTS). The application must be accompanied with the section of the company's quality manual dealing with automatic sampling. The section of the manual must include the following information:

a. the type of automatic seed sampler;
b. the procedures the company intends to use;
c. the name of the person responsible for the correct application of the conditions;
d. data indicating that the results obtained by this device are similar to other ISTA-approved manual sampling methods;
e. the data should include test results for percent purity, other seeds by number and percent germination tests of 10 seed lots. The same ten seed lots must be sampled by the automatic sampler and an ISTA-approved manual sampling method by an official seed sampler.

For approval, it is required that the results for each of the three quality traits of the composite samples should be within tolerances as provided in the ISTA rules in at least 70% of the 10 seed lots tested (7/10). If less than 70% of the seed lots results are not in tolerance, further seed lot sampling and testing must be conducted by the company to achieve at least 70%. If the application is acceptable, then the Canadian Food Inspection Agency (CFIA) must inspect the automatic seed sampling device at the establishment. Once SSTS provides a recommendation based on test data, approval of authorization is granted by the National Manager, Seed Section.

**Authorization Requirements:** If all conditions are fulfilled, the automatic seed sampler can be accredited/authorized. The approval, as well as the conditions of the approval, will be communicated in writing.

a. The automatic seed sampler must have an uninterrupted duct with the sampler container. The connection between the automatic seed sampler itself and the sample container must be constructed so that the seed cannot remain in the funnel or duct and seed cannot remain in the funnel or duct and seed be added or withdrawn;
b. All sections and parts of the automatic seed sampler must be constructed in such a way that they can be cleaned easily and thoroughly;
c. The minimum number of primary samples, as prescribed by the ISTA rules (sampling intensity), must be taken;
d. The proper adjustment and operation of the automatic seed sampler must be checked prior to operation;
e. The adjustment on the automatic seed sampler must be indicated and accessible to the seed sampler;
f. During the sampling of the same seed lot, the adjustment of the automatic seed sampler may not be altered;
g. The sample container must be identified in such a way that a direct link with the seed lot can be made;
h. Records of the use and maintenance of automatic seed samplers must be kept. These records should be maintained in a log book with at least the following information:
    - serial number of the composite sample container;
    - adjustments to the automatic seed sampler;
    - seed lot data (species, lot number and lot size);
    - size of the composite sample;
    - maintenance activities and date;
    - There must be clear operation instructions for the automatic seed sampler;
    - Any changes to the installation of the automatic seed sampling device must be documented.

**Post Authorization Requirements:** After approval of the automatic seed sampler to support the issuance of ISTA Orange International Seed Lot Certificates or for sampling under the automated entry processing (AEP), it must be checked at least once a year. The monitoring must be performed by the approved CFIA accredited laboratory or the official seed sampler. A comparison of test results from samples taken by the automatic seed sampler and those obtained from the samples taken by the ISTA-approved manual sampling method must be conducted by an official seed sampler and the Seed Science and Technology Section (SSTS) which is an ISTA member laboratory. The inspector retrieves samples from the automatic sampler and probed samples

and submits them to the SSTS for verification that the two sets of samples are in tolerance with each other.

In addition to taking samples, official seed samplers should inspect the company's records/books on the automatic sampler, at least annually, to verify that the company is:

a. documenting any changes to the installation of the automatic sampler, and
b. checking the functionality of the automatic sampler.

**Manual Stream Sampling**

An appropriate container must run through the entire cross section of the seed stream so that uniform primary samples are obtained. The container must not permit seed to enter and bounce out again. The sampler should draw primary samples at regular intervals. Sufficient primary samples need to be taken to verify that at least the minimum number of primary samples taken is that specified in Appendix II.

The container used for taking primary samples from the seed stream must be designed as follows:

a. the opening must be at least two times larger than the largest diameter of the seed;
b. the sides of the container must be high enough to prevent seeds from bouncing out;
c. it must be of sufficient length to enable the container to cut a complete cross section of the seed stream;
d. it must be large enough to prevent any overflow when taking a primary sample;
e. it must be such that it can be cleaned properly between seed lots.

**Other Equipment Requirements**

In addition to the approved trier, the following list of equipment may be required:

a. seals;
b. supply of suitable tape for patching openings made in the poly, cotton, or paper seed lot containers by the sampling trier;
c. pails (2-4) for collecting the primary samples or sheets of long clean paper, "nonstatic" stainless steel or metal seamless scoops, pans/pails are recommended;
d. Sample Submission Form;
e. note pad;
f. pocket light;
g. safety equipment, as necessary;
h. containers for submission of samples to the laboratory: cotton bags, or manilla envelopes, plastic bags for treated or inoculated seed.

i. moisture-proof containers for submission of samples for moisture determination (see Section 13.3.2.1).

**Cleaning and Care of Sampling Equipment**

All equipment used for sampling must be thoroughly cleaned before each use, and free from all extraneous matter including crop and weed seeds; disease bodies or spores; any seed parts, chaff, dust and inert foreign bodies; chemical residues such as seed treatments. Triers with residue seed could cause cross contamination of other seed lots or samples.

**For the Nobbe trier**: The more polished the inner surface of the trier is, the more freely the seed will flow. For the double sleeve trier, the rough edges and point of a sleeve trier should be occasionally dressed (removing sharp edges) with a file, emery or very fine sandpaper. This will greatly improve its use through jute or poly bags. The sleeves of the trier must fit together tightly. All triers must be undamaged with smooth bores and points. The method for cleaning will be based on the type of equipment and may be based the purpose and test for which the sample is to be drawn, i.e., treated seed or seed for disease tests.

Recommended cleaning methods are:

a. cleaning wipes;
b. cleaning solutions, such as hand or dish soap, citric acid, rubbing alcohol or water;
c. compressed air;
d. the use of bottle cleaning or gun cleaning tools.

**Methods for obtaining working samples:** Seed samples received in the seed Testing Laboratory in the seed testing laboratory (submitted sample) are required to be reduced to obtain working samples for carrying out various test.

**Mechanical method:** The reduction of sample size is carried out by the mechanical dividers suitable for all seeds except for chaffy and fuzzy seeds.

**Objective of mechanical dividing:**

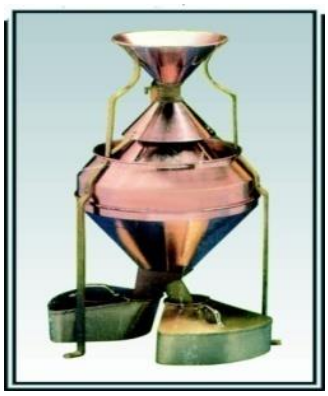
- To mix the seed sample and make homogenous as far as possible. To reduce the seed sample to the required size without any bias.
- The submitted sample can be thoroughly mixed by passing it through the divider to get 2 parts and passing the whole sample second time and 3rd time if necessary to make the seeds mixed and blended so as to get homogenous seed sample when the same seeds are passed through it into approximately equal parts.
- The sample is reduced to desired size by passing the seeds through the dividers repeatedly with one half remain at each occasion.

**Types of mechanical dividers:**

**a)Boerner divider:** It consists of a hopper, a cone and series of baffles directing the seeds into 2 spouts. The baffles are of equal size and equally spaced and every alternate one leading to one spout. They are arranged in circle and are directed inward. A valve at the base of the hopper retains the seeds in the hopper. When the valve is opened, the seeds fall by gravity over the cone where it is equally distributed and approximately equal quantity of seeds will be collected in each spout. A disadvantage of this divider is that it is difficult to check for cleanliness.

**b)Soil divider:** It is a sample divider built on the same principles as the Boerner divider. Here the channels are arranged in a straight row. It consists of a hopper with attached channels, a frame work to hold the hopper, two receiving pans and a pouring pan. It is suitable for large seeds and chaffy seeds.

**c) Centrifugal or Gamet divider:** The principle involved is the centrifugal force which is used for mixing and dividing the seeds. The seeds fall on a shallow rubber spinner which on rotation by an electric motor, throw out the seeds by centrifugal force. The circle or the area where the seeds fall is equally divided into two parts by a stationary baffle so that approximately equal quantities of seed will fall in each spout.



**Boerner divider**

**Soil divider**

**Centrifugal or Gamet divider**

**Random cup method:** This is the method suitable for seeds requiring working sample upto 10 grams provided that they are not extremely chaffy and do not bounce or roll (e.g.) *Brassica* spp. Six to eight small cups are placed at random on a tray. After a preliminary mixing the seed is poured uniformly over the tray. The seeds that fall into the cup is taken as the working sample.

**Modified halving method:** The apparatus consists of a tray into which is fitted a grid of equal sized cubical cups 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 method until a working sample size is obtained.

**Spoon method:** This is suitable for samples of single small seeded species. A tray, spatula and a spoon with a straight edge are required. After preliminary mixing, the seed is poured evenly over the tray. The tray should not be shaked thereafter. With the spoon in one hand, the spatula in the other and using both small portions of seed from not less than 5 random places on the tray should be removed. Sufficient portions of seed are taken to estimate a working sample approximately but not less than the required size.

**Hand halving method:** This method is restricted to the chaffy seeds. The seed is poured evenly on to a smooth clean surface and thoroughly mixed into a mound. The mound is then divided into 1/2 and each half is mound again and halved into 4 portions. Each of the 4 portions is halved again giving 8 portions. The halved portions are arranged in rows and alternate portions are combined and retained. The process is repeated until the sample of required weight is obtained.

**How to weight of working sample:** When a seed annalists taking weight of working sample then he should taking the care of the weight measuring unit and use four decimal weighing balance.

| Weight (gm) | Number of decimal places | Example |
|---|---|---|
| Less than 1 | 4 | 0.7534 |
| 1 to 9.999 | 3 | 7.534 |
| 10 to 99.99 | 2 | 75.34 |
| 100 to 999.9 | 1 | 753.4 |
| 1000 or more | 0 | 7534 |

**Instructions for sending samples**

- Pre-requisite in sampling is that the seed lot received in containers / bags must be properly sealed and marked for identification with a single lot designation.
- At the time of sampling, all the samples drawn must bear identification corresponding to that of the lot certificate.
- The sampler should seal or supervise the sealing of the sample container / bags after drawing sample.
- After taking samples which may be more than required for seed testing purpose, a through mixing of the samples is to be done.
- Divide it using a seed divider and then the required amount should be submitted to the seed testing laboratory after putting proper identification mark.

- If mechanical divider is not available at the spot, a representative sample should be obtained by putting the entire quantity of seed on a clean floor, mixing properly and halving the sample until the desired quantity is obtained.
- For moisture determination, 100g of seeds for species which need grinding and 50g for all other species. Sample should be submitted in an air-tight container, like polythene bags of 700 guage or glass bottle with tight cap to the laboratory.

**Quantity and dispatch of sample for testing**

**Weight of submitted sample:** The minimum weight for submitted samples for various tests are as follows.

**1. Moisture test:** 100 g for those species that have to be ground and 50 g for all other species.

**2. For verification of species and cultivar**

| **Crop** | **Lab only (g)** | **Field plot & Lab (g)** |
|---|---|---|
| Peas, beans, maize, soybean and crop seeds of similar size | 1000 | 2000 |
| Barley, oats, wheat and crop seeds of similar size | 500 | 1000 |
| Beet root and seeds of similar size | 200 | 500 |
| All other genera | 100 | 250 |

**Dispatch of submitted sample**

1. Each submitted sample should be sealed and marked
2. The label should contain all the necessary details such as variety, class of seed, quantity in the lot, to whom it belongs, name of the producer, seed treatment, date of harvesting and threshing if known, sampled by, date of sampling and the kind of tests required.
3. After marking the sample, it should be packed so as to prevent damage during transit. For germination test sample should be packed preferably in cloth bag, for moisture content determination, sample should be packed separately in moisture proof containers.
4. Samples should be despatched by the sampler to the seed testing laboratory without delay.

**Types of sample used in Seed Testing Laboratory (STL)**

**Service sample:** Sample received from other than seed certification agencies and seed inspectors

**Certified sample:** Sample received from certification agencies or officers

**Official sample:** Sample received from the seed inspectors.

**LIST OF STATE SEED CERTIFICATION AGENCY**

1. Andhra Pradesh: Andhra Pradesh State Seed Certification Agency.
2. Assam: Assam State Seed Certification Agency,
3. Bihar: Bihar State Seed Certification Agency,
4. Chhattisgarh: Chhattisgarh State Seed Certification Agency,
5. Delhi: Delhi Seed Certification Unit
6. Gujarat: Gujarat State Seed Certification Agency,
7. Haryana: Haryana State Seed Certification Agency,
8. Himachal Pradesh: Himachal Pradesh State Seed Certification Agency
9. Jammu & Kashmir: (a) Seed Certification Wing Department of Agriculture Jammu Division, Talab Tilloo Jammu
   (b). Seed Certification Wing Department of Agriculture Kashmir Division, Lalmandi, Post Office Jawahar Nagar Srinagar-190008
10. Karnataka: Karnataka State Seed Certification Agency,
11. Kerala: Kerala Department of Seed Certification Directorate of Agriculture,
12. Madhya Pradesh: Madhya Pradesh State Seed Certification Agency,
13. Maharasthra: Maharashtra State Seed Certification Agency,
14. Orissa: Orissa State Seed Certification Agency,
15. Puducherry: Puducherry State Seed Certification Agency
16. Punjab: Punjab State Seed Certification Authority,
17. Rajasthan: Rajasthan State Seed & Organic Production Certification Agency,
18. Sikkim:Sikkim Seed Certification Wing Department of Agriculture Government of Sikkim
19. Tamil Nadu: Tamil Nadu Department of Seed Certification
20. Uttar Pradesh: Uttar Pradesh State Seed Certification Agency,
21. West Bengal: West Bengal State Seed Certification Agency
22. Uttarakhand: UttarakhandState Seed and Organic Production Certification Agency
23. Telangana: Telangana State Seed Certification Agency

# PRINCIPLES AND PROCEDURES OF PHYSICAL PURITY ANALYSIS

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## Introduction

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

1. Improving the plant stand (by increasing the pure seed component).
2. Raising a pure crop (by eliminating other crop seed and weed seeds).
3. Raising a disease free-crop (by eliminating inert matter).
4. In the use of seed drill (by selecting uniform particles).

There is a need for physical purity analysis for:

1. Seed Certification or Seed Law Enforcement Agencies to judge that the seed lot conforms to the prescribed standards.
2. Seed processing plants for using right kind of processing equipment.
3. Physical purity analysis is a pre-requisite for germination test because 'pure seed' component is used for germination testing.

## Objective

The primary objective of physical purity analysis is to determine:

1. The percentage composition by weight of the sample being tested and by inference the composition of seed lot; and
2. The identity of various species of seeds and inert particles constituting the sample.

The definition of the various physical purity components in the ISTA Rules are as follows:

## Pure seed

The pure seed shall refer to the species stated by the sender, or found to predominate in the test, and shall include all botanical varieties and cultivars of that species (even if immature, undersized, 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 etc.) 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 follows:

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*.
   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 caryopsis 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 pratensis* and *Dactylis glomerata*.
   d. Multiple seed unit's (MSU) are left intact in the pure seed fraction e.g. *Dactylis and Festuca*.
   *e.* Attached sterile florets are not removed, but left attached 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 seed**

Other crop seed 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 seed**

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

**Inert matter**

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

**General principles**

As per ISTA Rules, the working sample is separated into three components i.e. pure seeds, other seeds, and inert matter. The percentage of each part is determined by weight. All species of seed and each kind of inert matter present

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:

a. Dividers
    1. Soil type
    2. Boerner (work on gravitational force)
    3. Gamete (works on centrifugal force and electrically operated)
b. *Balance*: Electric or electronic balance are better due to their accuracy and quickness
c. Blowers
d. Diaphnoscope using reflected light are used to separate inert matter such as empty florets of grasses.
e. Sieves
f. Sample pans, dishes, forceps, spatula and hand lens
g. Seed herbarium of crop and weed seed

**Procedure**

*Obtaining working sample:* Since the size of the working sample is minute as compared with the size of the seed lot to which it represent, it is therefore, very essential that the working sample should be obtained in accordance with the procedures. The working sample shall be either a weight estimated to contain atleast 2,500 seed units or not less than weight indicated i.e. 40 g for *Oryza sativa*. Boerner or soil type seed divider should be used to homogenize the submitted sample before reducing it to the size of working sample. The following guidelines need to be followed:

a. Check the cleanliness of the divider and the container.
b. Pour the entire contents of the submitted sample into the hopper of the divider.
c. Allow the 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.
d. Recombine the contents of both sample receiving pans and again pass it through the divider.
e. Repeat this process twice in order to homogenize the submitted sample.
f. Divide the submitted sample.
g. Set aside the contents of one container.

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

***Separation***

1. Clean the work board, sample and purity dishes before starting the separation
2. Examine the working sample to determine the use of particular aid such as blower or sieves for making separation.
3. After preliminary separation with the help of sieves or blower, place and spread the retained or heavier portion (A) on the purity work board.
4. With the help of spatula or forcep, draw working sample into thin line and examine each particle individually. The criteria used being the external appearance (shape, size, colour, gloss, surface texture) and/on appearance in transmitted light.
5. 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.
6. Seed enclosed in fruits other than those indicated in pure seed should be separated and the detached empty fruit/appendages classed as inert matter.
7. Collect the pure seed in the sample pan.
8. 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).
9. 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.
10. Weight each component, pure seed, other crop seed, weed seed and inert matter in grams to the number of decimal places shown below:

| S.No. | Wt. of working sample (g) | No. of decimal place required | Example |
|---|---|---|---|
| 1. | Less than 1 | 4 | 0.9025 |
| 2. | 1 to 9.990 | 3 | 9.025 |
| 3. | 10 to 99.99 | 2 | 90.25 |
| 4. | 100 to 999.9 | 1 | 902.5 |
| 5. | 1000 or more | 0 | 9025 |

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

*Reporting results:* The results of purity test be given to one decimal place only and the percentage of all component must total 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.

**Definition of pure seed**

***Gramineae***

***Oryza* (Paddy)**

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

   *N.B: Seeds with awns longer than length of floret are reported according Rule 3.7.*

***Hordeum* (Barley)**

1. Floret, with lemma and palea enclosing a caryopsis, with or without awn or with or without rachis segment irrespective of their length.
2. Piece of floret containing a caryopsis larger than one-half the original size.
3. Caryopsis
4. Piece of caryopsis larger than one-half the original size.

   *N.B. Florets with awn or rachis segment longer than length of floret are reported according to Rule 3.7*

***Avena* (Oat)**

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

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

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

***Panicum* (Sawa)**

1. Spikelet, with glumes, lemma and palea enclosing a caryopsis, plus attached sterile lemma.
2. Floret with lemma and palea enclosing a caryopsis.
3. Caryopsis
4. Piece of caryopsis larger than one-half the original size.
5. No need to check the presence of caryopsis.

***Pennisetum* (Pearl millet)**

a. Panicle of 1-5 spikelets (spikelets with glumes, lemma and palea enclosing a caryopsis, plus attached sterile lemma) with involucre of bristles.
b. Floret with lemma and palea enclosing a caryopsis.
c. Caryopsis.
d. Piece of caryopsis larger than one-half the original size.

***Sorghum* (Jowar)**

1. Spikelet, with glumes, lemma and palea enclosing a caryopsis with or without hyaline palea or lemmas, rachis segments, pedicel(s), awn(s), attached sterile or fertile floret(s).
2. Floret, with lemma and palea, with or without awn.
3. Caryopsis.
4. Piece of caryopsis larger than one-half the original size.

***Leguminaceae*** (Gram, Pea, Mung, Urd, Bean, Cluster bean, Soybean, Lupins, *Crotolaria* (Sunhemp), *Medicago, Arachis, Trifolium*); *Cruciferae* (Radish, Mustard, Cabbage and Cauliflower)

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

***Solanaceae*** (Chillies, Brinjal, Tomato, Tobacco); *Linaceae* (Linum); *Liliaceae* (Onion, Garlic); *Amaranthaceae* (Amaranthus); *Cucurbitaceae* (Watermelon, Longmelon, Muskmelon, Cucumber, Pumpkin, Squash, Bottle guard); *Pedaliaceae* (Sesamum)

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

***Umbelliferae*** (*Carum*, Coriander, Cumin, Carrot, Fennel)

- Schizocarp is a dry fruit which separates into two or 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.

*N.B. Fruits with pieces of pedicel longer than the length of schizocarp/mericarp are reported according to Rule 3.7*

***Malvaceae*** (Cotton)

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

***Compositeae*** **(**Sunflower, Lettuce, Chicory)

- 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 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% of 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.

**Physical Purity: Evaluation Sheet**

<table>
<tr><td></td><td colspan="5">INTERNATIONAL COURSE ON SEED PRODUCTION AND QUALITY EVALUATION<br>GROUP ...<br>SPECIES NAME: PURITY TEST RESULTS DATE: JANUARY 2018</td></tr>
<tr><td>Group No.</td><td>Initial Weight</td><td>Pure Seed</td><td>Other Seed</td><td>Inert Matter</td><td colspan="2">Weight of Components</td></tr>
<tr><td>[g]</td><td></td><td></td><td></td><td></td><td colspan="2"></td></tr>
<tr><td>[%]</td><td></td><td></td><td></td><td></td><td colspan="2"></td></tr>
<tr><td rowspan="4">Number and Botanical Name of Other Seeds</td><td colspan="4">Species Name</td><td colspan="2">Number</td></tr>
<tr><td colspan="4"></td><td colspan="2"></td></tr>
<tr><td colspan="4"></td><td colspan="2"></td></tr>
<tr><td colspan="4"></td><td colspan="2"></td></tr>
<tr><td>Kind of Inert Matter</td><td colspan="6"></td></tr>
</table>

# INTRODUCTION TO GENETIC PURITY TESTING: IMPORTANCE, PRINCIPLES, TOOLS AND ESSENTIAL PROTOCOLS

**Sherry Rachel Jacob[2] and M.B. Arun Kumar[1*]**

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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 variety.

The term 'Genetic purity/ Cultivar purity' generally infers that plant population of a particular variety is homogenous and genetically identical i.e. true to the 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 varieties should be ensured for their identity and genetic purity before selling it to farmers.

## Objective of genetic purity testing

At International Seed Testing Laboratories, seed lots are subjected to genetic purity testing to ensure to what extent the said seed lot conforms to variety/species mentioned on its label or in simple words it ensures the identity of seed. Accordingly, the objective of cultivar purity testing as given in International Seed Testing Rules 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. In the given objective term 'species' is included because in many grass seeds like rye grass, verification of seed lot is done at species level. Where as in India, seed lot is subjected to genetic purity testing to ensure whether lot meets the minimum requirements of genetic purity standards as given in Indian Minimum Seed Certification Standards for respective crops. Accordingly, the objective of cultivar purity testing as given in Indian Minimum Seed Certification Standards is to determine the genetic purity of a given seed lot of a released cultivar and the extent to which the submitted sample conforms to the prescribed standards.

**Field of application**

A genetic purity test result of any seed lot is considered to be valid, only if the species or cultivar is stated by the sender of the sample and if an authentic standard sample of the species or cultivar was used during genetic purity test for comparison. The characters compared may be morphological, physiological, cytological or chemical.

**Methods to assess genetic purity**

- Examination of seeds:
- Examination of seedlings:
  1. Coleoptiles pigmentation
  2. Seedling colour test in *Beta spp.*
  3. Colour of cotyledons in *Brassica spp.*
  4. Fluorescence test in *Lolium spp* and *Festuca 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*

*AOSA: Association of Official Seed Analysts

**General Principle of Genetic Purity Testing:**

For any of the genetic purity testing methods mentioned above, the principle of the procedure is the same. As per the method to be adopted, the working sample of definite size is drawn from the submitted sample and individual seed/seedling/plant characteristics are compared with that of from an authentic sample. Normally, seeds are compared with seeds from the authentic sample and seedlings/plants are compared with seedlings/plants at the same stage of development, grown from the authentic sample contemporaneously, near-by and in identical environmental conditions. Number of seeds/seedlings/plants that are deviating with respect to any of the character/s

of an authentic sample will be considered off type/of the other variety and will be counted. The result of genetic purity will be expressed in percentage of number of seeds/seedlings/plants that are true to the type to the total number of seeds/seedlings/plants subjected to the test.

As per International Seed Testing Association (ISTA), 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. But as per Indian Minimum Seed Certification Standards, inclusion of one authentic sample for every 10 submitted samples would serve the purpose. 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 judgement is expressed as to the conformity of the sample under test.

**Submitted sample:**

As per ISTA, for verification of cultivar/species, a separate submitted sample of prescribed size (as given below) should be sent to the seed-testing lab.

| | Field plot only (g) | Laboratory and Field plot (g) |
|---|---|---|
| Pisum, Phaseolus, Vicia, Lupinus, Zea, Glycine and species of other genera with seeds of similar size | 1000 | 2000 |
| *Hordeum, Avena, Triticum*, *Secale*, and species of other genera with seeds of similar size | 500 | 1000 |
| Beta and species of other genera with seeds of similar size | 250 | 500 |
| All other genera | 100 | 250 |

Under Indian Minimum Seed Certification Standards, conditions for submission of submitted sample is same as that of ISTA's requirement for Field plot only except for tubers/planting stakes/roots/corms, where submitted sample size is 250 numbers.

**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 i.e., sequential testing using a batches of 50 seeds. 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.

(d) For electrophoretic characteristics, the developed electrophoretic profile of proteins/isozymes from test sample will be compared with that of authentic sample. Based on presence or absence of bands in the profile, off-types/deviants will be identified.

**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:

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"

**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. It is well known that "all inherent morphological manifestation of variety differences must ultimately have a biochemical difference but not all biochemical differences are necessarily reflected morphologically". With biochemical differences being more numerous than morphological differences, each truly distinct cultivar should posses' correspondingly distinct biochemical characteristics. This confirms the broad scope of utilization of biochemical tests in variety identification and purity testing. Application of such 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

Figure 1: Seed Coat Phenol Colour Reaction in wheat

(ISTA) has recommended this test for ensuring the genuineness of wheat cultivars.

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

**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.

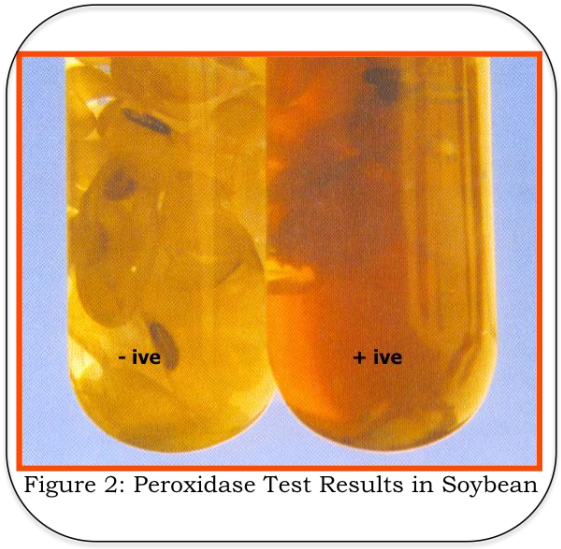


Figure 2: Peroxidase Test Results in Soybean

**Electrophoresis Techniques**

In this technique, proteins/Isoenzymes are separated into distinct bands in a support medium of polyacrylamide or starch gel under the influence of electric current applied across the medium. The separation is due to the differences in the size/charge/both of the protein/isoenzyme involved. The difference between the varieties is established based on presence or absence of a particular protein/isozyme band at a particular position in a support medium, which is marked by Relative mobility (Rm) value of that particular band.

***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. Isozymes do not suffer the limitations of morphological characters, such as variations due to environmental fluctuations, need of growing plants till maturity, limited range of expressions etc. 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. Electrophoretic technique is profusely used in bread industry to ensure the identity of wheat grains. Gliadin and glutenins from specific loci are important in deciding the bread/pasta making quality of wheat varieties and bread industry personnel rely on electrophoretic profile results in selection of wheat varieties for bread making purpose as well as for ensuring the identity of wheat grain lots. Like any other biochemical tests, electrophoretic technique alone is not sufficient to identify the extent of admixtures when the admixture varieties' have the similar electrophoretic profile as that of the variety under question. In that case it is necessary to use other tests along with electrophoretic techniques in sequential fashion. Even though electophoretic 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**

In recent past, DNA based markers have been applied for ensuring the genetic purity of seed lots, 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 ($^{32}P$) 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

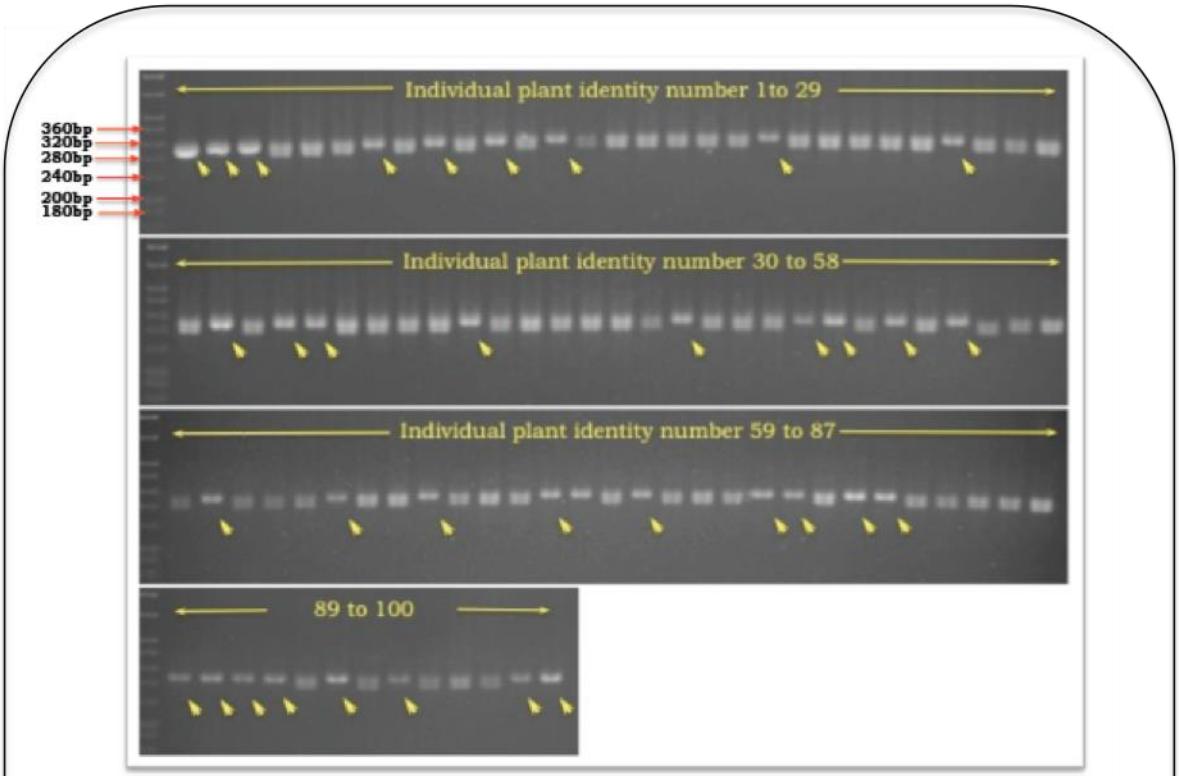
as RAPDs, amplified fragment length polymorphisms or microsatellites (also called simple sequence repeats (SSRs) are more cost-effective than RFLPs and faster but are subject to the relatively high expense of thermostable polymerases and the time, personnel and space needed to run and score numerous 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 $F_1$ 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. Some of these problems can be alleviated by cloning and sequencing RAPD products, redesigning longer PCR primers and then converting these random markers to Sequence-Characterized Amplified Regions (SCAR) marker. However, the effort and expense required for doing these eliminates the major advantage of RAPD technology.

***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




Assessment of genetic purity in brinjal hybrid seeds using the SSR marker. M=20 bp DNA Ruler from GeNie™, Lane 1–100 = individual $F_1$ seeds. Lane 1, 2, 3, 7, 9, 11, 13, 21, 26, 31, 33, 34, 39, 46, 50, 51, 53, 55, 60, 64, 67, 71, 72,74, 78, 79, 81, 82, 88, 89, 90, 91, 93, 95, 99 and 100 (arrow) represents a selfed plant, a contaminant

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.

**Testing Genetic Purity of GM Seeds**

Farmers could able to harness the full potential of GM variety only when he gets the genetically pure seeds of that variety. Apart from this, the timely availability of genetically pure GM seeds will also helps in faster acceptance and dissemination of GM technology among the farmers. So it is necessary to ensure the varietal purity of GM seeds reaching the farmers through proper seed quality control system. Testing the varietal purity of GM seeds will be as similar to that of testing the varietal purity of any conventional variety. But in case of GM variety the purity has to be confirmed with respect to both the expression of transgene and varietal characters of the particular variety. This demands the special techniques to confirm the expression/presence of transgene in GM variety.

**Analytical Assays for GMO Detection**

GMOs can be identified by detecting either the inserted genetic material at DNA level, the mRNA transcribed from the newly introduced gene, the resulting protein, the metabolite or the phenotype. The analytical tests on raw materials (*e.g.* seeds) are generally carried out with the polymerase chain reaction (PCR method) detecting the inserted DNA, immunological assays detecting the resulting protein (*e.g.* the enzyme-linked immunoassay (ELISA) and lateral flow sticks), or using bioassays to detect the resultant phenotype (*e.g.* herbicide bioassays). Herbicideq bioassays and immunoassays can be regarded as "low-technology methods" because they can be set up in most laboratories while PCR and microarrays are regarded as "high-technology methods" requiring more equipment and trained specialists.

***Phenotypic Bioassays (Herbicide Bioassays)*:** Phenotypic characterization allows detection of the presence or absence of a specific trait. So far only tests for traits such as resistance/tolerance to herbicides are available, which are termed as 'herbicide bioassays', which consist of conducting germination tests on solid germination media in the presence of a specific herbicide, where non-GM and GM seeds show distinct characteristics. The detection level is dependent on germination of the seed and the germination methods should ensure that all viable seeds of the tested sample germinate. Seeds tested positive should be exposed to subsequent tests for confirmation. At present,

herbicide bioassays are available for Roundup Ready soybean, maize, cotton and oilseed rape, and Liberty Link maize. The herbicide bioassay tests are claimed to be accurate, inexpensive, and useful as a preventative test primarily for seed companies. Companies are using the herbicide bioassays to check individual shipments as a quality assurance program.

***Protein-based Methods:*** Immunoassay is the current method for detection and quantification of new (foreign) proteins introduced through genetic modification of plants. Making a valid identification of the foreign protein in GM seeds/plants using immunoassays depends on the availability of the particular proteins for development of the antibodies, which is the essence of the assay. The proteins can be proprietary of the company who developed the plant variety and thus not commonly available. Furthermore, the likelihood of development of a successful immunoassay depends on certain characteristics of the antigen used for development of the antibody, *i.e.* size, hydrophobicity and the tertiary structure of the antigen. The specificity of the antibodies must be checked carefully to elucidate any cross-reactivity with similar substances, which might cause false positive results. Factors such as quality and sensitivity of kits, threshold limits, work environment, extraction efficiency, and ability to distinguish between close values will affect optimization and validation of the tests.

***DNA-based Methods:*** DNA-based tests use the polymerase chain reaction (PCR) to detect specific DNA sequences. PCR tests can be designed to detect any of the inserted genetic material: promoter, structural gene, stop signal or marker gene. The exact design of any particular test depends on the requirements. PCR can be used for a general screening of GMOs using primers that recognize common DNA, which most GMOs harbour, for example the commonly used Cauliflower Mosaic Virus (CaMV) 35S promoter or *Agrobacterium tumefasciens nos* promoter, or the *nos* terminator.

PCR can also be used to detect and identify specific GMOs more precisely. However, making a valid and unique identification by using PCR requires information about the inserted sequences. For a positive unique identification it should be related to the specific transformation event of the GMO. The only unequivocal strategy is to use plant-construct junction sequences as primer targets. This gives indirect information of the whole inserted sequence including the used promoter, active gene and enhancer/ terminator. There are several commercial sources of PCR test kits as well as companies that offer GMO testing of samples.

# GERMINATION TESTING: PRINCIPLES AND PROCEDURES

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The purpose of laboratory testing of seed germination is to assess seed quality or viability and to predict performance of the seed and seedling in the field. A notified laboratory under Seeds Act (1966) or qualified laboratory of ISTA for testing seeds must test seed processed for sale. The ultimate aim of testing the germination in seed testing laboratory is to obtain information about the planting value of the seed sample and by inference the quality of the seed lot. In addition, the laboratory germination results are also required for comparing the performance potential or superiority of the different seed lots. In general, the farmers, seeds men and public agencies use the germination results for the following purposes:
Sowing purposes, with a view to decide the seed rate to achieve desired field establishment.

- Labeling purposes.
- Seed certification purposes.
- Seed Act and Law Enforcement purposes.

In seed testing germination has been defined as "the emergence and development from the seed embryo of those essential structures which, for the kind of seed tested indicate its ability to develop into a normal plant under favourable, conditions in soil". The seedlings devoid of an essential structure; showing weak or unbalanced development; decay or damage affecting the normal development of seedling are not considered in calculating the germination percentage. Factors that can affect the performance of seed in germination tests include; diseased seed, old seed, mechanically damaged seed, seed stored under high moisture, and excessive heating of seed during storage or drying.
In most cases a seed treatment will improve germination of seed only if the poor quality is due to seed-borne disease. Several different kinds of testing are available depending on the type of seed to be tested, the conditions of the test, and the potential uses of the seed. The most common tests are the cold germination test, accelerated aging test, the tetrazolium test and warm germination test. Each test is designed to evaluate various qualities of the seed.

## Germination Testing

The most common test is a warm germination test because it is required by seed laws to appear on the label. The percentage of germinating seed in a warm germination test must be printed on the label of the seed if it is to be sold as seed. The warm germination test reflects the field emergence potential of a seed

lot under ideal planting conditions. Usually 400 seed from each seed lot are placed under moist conditions on blotters, rolled towels, or sand and maintained at about 75 to 85 degrees F for about seven days in most of the cases. At the end of this period the seedlings are categorized as normal, abnormal, or diseased, and dead or hard seeds. The percentage germination is calculated from the number of normal seedlings from the total number of seeds evaluated. The method of testing germination is discussed below.

The first and foremost step is to draw a true representative sample from the seed lot. To obtain a random sample for testing it is always best to take samples from different parts of the bag or container. If the seed to be tested is from a seed lot that contains more than one bag, samples must be taken from several bags. A good rule of thumb for determining how many bags to sample is to take samples from a number of bags that represents the square root of the lot size. For example if the lot contains nine bags, then sample at least three bags. If the lot contains 100 bags, then sample at least 10 bags. The sample thus drawn is further divided and the required numbers of seeds are the taken to perform the actual test.

***Essential equipments and supplies for germination test:***

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

***a) Seed Germinator:*** The seed germinators are the essential requirement for germination testing for maintaining the specific conditions of temperature, relative humidity and light. The seed germinators are generally of two types, namely: Cabinet germinator and walk in germinator. The cabinet seed germinators are essential under the situations, where various kinds of seeds that require different sets of conditions, are being handled in the laboratory. The number of the pieces of the germinators required by the laboratory will depend on the number of seed samples and the species being analysed by the laboratory. The seed testing laboratories that handle large number of seed samples and require maintaining only fewer (2-3) sets of temperature conditions, the walk-in-germinators are preferred. Such germinators are more useful for conducting the germination tests in sand media, which require large germination space.

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

***c) Other equipment:*** The other equipment required for germination testing include the refrigerators, scarifier, hot water bath, incubator, forceps, spatula,

germination, boxes, plastic plates, roll- towel stands and plastic or surgical trays, etc. A large oven with temp. Range 100 -200 C is also required for sterilizing the sand.

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

**Care of equipment:** The seed analyst must ensure that,

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

**Handling of substrata**

The accuracy and reproducibility of the germination result are very much dependent on the quality of the substrata (paper and sand) used for germination testing. The germination substrata must meet the following basic requirements:

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

***a) Paper substrata:*** The paper substrata are used in the form of top of paper (TP) or between paper (BP) tests. In most of the laboratories, paper-toweling method (Roll towel test) is most commonly used for medium sized and bold seeds. The paper substrata are not reusable.

***b) Sand substrata:*** The sand substrata have advantage of being relatively less expensive and reusable. The results in sand media are more accurate and reproducible in comparison with 'roll towel' tests especially in case of seed lots that are aged or heavily treated with chemicals. The sand should be reasonably uniform and free from very small and large particles. It should not contain toxic substances and its pH should be within the range of 6.0- 7.5. The sand should be washed, sterilized and graded with a sieve set having holes of 0.8 mm diameter (upper sieve) and 0.05 mm diameter (bottom sieve). The sand retained on the bottom sieve should only be used.

**Testing of Substrata**

***Phytotoxicity:***

- The substrata should be tested for its phytotoxicity, capillary rise, moisture holding capacity and bursting strength, etc., before accepting the supplies in the laboratory. Periodic checks of the quality of the substrata should also be made in the laboratory.
- By germinating the seeds of Brassica, Onion, Chillies or Berseem and studying the phytotoxic symptoms on the germinating seedlings can check the phytotoxicity of the paper or sand substrata.
- The paper should be cut into circles and rectangles or squares of the desired size according to the size and shape of the containers.
- Place 2-4 circles/rectangles of the paper to be tested in the petridishes or plastic containers.
- Moisten the paper with tap water using only enough water to saturate the paper. Excess water should not be used.
- Arrange 25 seeds of Brassica, Onion, Chillies or Berseem properly spread over the moist paper.
- Cover the dishes with lids.
- Conduct a control test as outlined above (step 1-4) using paper of accepted quality such as 'Waterman' or 'Sunlit' brand filter paper.
- Transfer the test to the prescribed temperature conditions of the species used at test crop.
- Evaluate the test 1-2 days before the date of the first count of the crop specified
- Check the phytotoxic symptoms on the seedlings.
- Compare the seedlings with those grown on the non-toxic paper (control test).

- The phytotoxic symptoms include shortened roots; discolored root tips; root raised from the paper; inhibition of root hairs development and root hairs bunched.
- The symptoms are more pronounced at an early stage of root growth.
- The phytotoxic symptoms are also evident in the plumular areas in the form of thickened or flattened plumules or, coleoptiles.
- The phytotoxicity of the sand substrata can also be measured by the procedure outlined as above. However, care need to be exercised that the sand substrata should be moistened with the measured quantity of the water and the seeds are planted on the top of sand (TS).

The pH of the substrata can be measured with the help of pH paper or pH meter as follow:

- Soak the paper or sand in water for 16-18 hrs.
- Decant the water.
- Measure the pH with litmus paper or pH meter.

If the, sand substrata are found to be acidic or alkaline, wash it thoroughly with the water and sterilize before use.

***Capillary Rise:***

- Cut four strips of germination paper 10 mm wide; two in machine direction and the other two in cross machine direction.
- Take distilled water in small glass beakers.
- Immerse one end of each strip in the water to a depth of 20 mm.
- Wait for 2 minutes and then measure the height to which water has risen in the strip to the nearest mm.
- Commute the average for the two strips cut in machine direction or cross machine direction separately.
- The lower value of the two averages should be considered as capillary rise.

***Bursting strength:*** The bursting strength of the paper is measured with equipment; however, it can be checked as follows:

- Hold the two ends of the germination paper and exert the pressure by stretching the paper with mid force.
- Soak the paper in water for 1-2 hours.
- The paper of desired bursting strength would not tear off easily.

**Test conditions**

**a) Moisture and aeration:** The moisture requirements of the seed will vary according to its kind. Large seeded species require more water than the small seeded species. It is essential that the substratum must be kept moist

throughout the germination period. Care need to be taken that the sub-stratum should not be, too moist. The excessive moisture will restrict the aeration and may cause the rotting of the seedlings or development of watery seedlings. Except the situations where a high moisture level is recommended (e.g. Paddy and jute), the substratum should not be so wet that a film of water forms around the seeds. In situations where low level of moisture is recommended (e.g. Cucurbitaceous seeds), the moist substratum should be pressed against the dry blotters or towel paper, to remove excess moisture. The water used for moistening the substratum must be free from organic and inorganic impurities. Normally the tap water is used. However, it is essential to measure the pH .of water before its use. The pH of the water should be in the range of 6.5- 7.5. Under the situations where pH of the water is not satisfactory, distilled water or deionized water may be used. Under such situation care need to be exercised to aerate the tests frequently to provide oxygen supply to the germinating seedlings because oxygen level in distilled water is very low. The initial quantity of water to be added to the substratum will also depend on its nature and dimensions. Subsequent watering, if, any may be left to the discretion of the analyst but it should be avoided as far as possible because it may cause the variation in germination results. In order to reduce the need for additional watering during the germination period, the relative humidity of the air surrounding the seeds should be kept at 90-95 % to prevent loss of water by evaporation. Special measures for aeration are not usually necessary in case of top of paper (TP) tests. However, in case of 'Roll towel' tests (BP) care should be taken that the rolls should be loose enough to allow the presence of sufficient air around the seeds. In case of sand media, the sand should not be compressed while covering the seeds.

**b) Temperature:** The temperature is one of the most important and critical factors for the laboratory germination tests. The temperature requirement for germination is specific according to the kind of crop or species. This can vary within the species and with the age of seeds. At very low or high temperatures, the germination is prevented to a larger extent. The temperature should be uniform through out the germinator and the germination period. The variation in temperature inside the germinator should not be more than 1°C. The prescribed temperature for germination of agricultural, vegetable or horticultural seeds, provided in the Rules for Seed Testing can be broadly is classified into two groups, viz. constant temperatures and alternate temperatures.

*Constant temperature:* Wherever, the constant temperatures are prescribed or recommended for the germination tests, the tests must be held at the specific temperature during the entire germination period.

*Alternate temperature:* Wherever, the alternating- temperatures are prescribed, the lower temperature should be maintained for 16 hours and the higher for 8 hours; a gradual changeover lasting 3 hours is usually satisfactory for non-

dormant seeds. However, a sharp change over lasting 1 hour or less, or transfer of test to another germinator at lower temperature, may be necessary for seeds, which are likely to be dormant.

**c) Light:** Seeds of most of the species can germinate, in light or darkness. It is always better to illuminate the tests for the proper growth of the seedlings. Under the situations where light is essential for germination, tests should be exposed to the natural or "artificial source of light. However" care must be made to ensure that an even intensity is obtained over the entire substrate, and that any heating from the source does not affect the prescribed temperature. Seeds that require light for germination must be illuminated with cool fluorescent light for at least 8 hours in every 24 hours cycle. Under the situation where testing of the seed is required to be undertaken at alternating" temperatures together with light, the tests should be illuminated during high temperature period.

**Laboratory procedures**

The working sample for germination test consists of 400 pure seeds randomly drawn either manually or with the help of counting devices. The seed for germination test must be drawn as follows in accordance with the following two situations:

- When both purity and germination tests are required.
- Seeds for germination tests must be taken from the pure seed fraction after conducting the physical purity analysis. The counting of the seed must be made without discrimination as to the size and appearance.
- Only germination test is required.
- If, the percentage of pure seed is estimated or determined to be above 98 per cent, the pure seed for germination test shall be taken indiscriminately from a representative portion of the submitted sample;
- If, the pure seed is found to be less than 98 percent, the seeds for germination test must be obtained by separating the sample into two components, namely
- The pure seed and Seeds of other species and inert matter.
- For this purpose, at least one-fourth of the quantity required for regular purity analysis must be used after proper mixing and dividing the submitted sample.

**Number of Replications:**

Four replication of 100 seeds, A minimum of 3 replication of l00 seeds may be used under unavoidable situations or Eight or six replications of 50 seeds or Sixteen/twelve replication of 25 seeds according to the kind of and size of containers.

**Paper Substrata:**

- Check the quality of germination paper before accepting the supplies.
- Measure the pH, capillary rise, bursting strength and phytotoxicity of the germination paper.
- Store the germination paper under hygienic conditions and protect it from dust and Micro flora.
- Very old stock should not be used as they often get contaminated. Such paper usually shows phytotoxic symptoms.

***Between Paper (BP) Media (Roll Towel Test):***

- Soak the towel paper in water.
- Remove the water.
- Wash the paper with running water.
- Remove extra moisture by pressing the soaked paper by hand and holding it in plastic/surgical trays placed on the tabletop in slanting position.
- Place two layers of wet paper toweling as substratum.
- Check Test number provided on the Analysis Card sample and label tally each other.
- Record the test number, crop and date of putting on the wax paper or tag.
- Arrange seeds spaced properly.
- Place one layer of wet towel paper over the seed.
- Turn up two inches of the bottom edge.
- Roll firmly from left to right and secure with rubber band in the center.
- Place the prepared roll towel in roll towel stand or baskets.
- Transfer the basket or roll towel stand in the germinator maintained at the desired temperature.

***Top of Paper (TP) Media:***

- Paper of known quality such as 'Sunlit' or 'Whatman' filter paper should be used.
- Crepe Kraft (towel) paper or blotter paper of unknown quality should not be used for top of paper tests.
- The paper should be cut in the form of circles/squares or rectangles according to the size and shape of petridish/container.
- Put 2-3 layer of filter paper in the petridish/ germination box having airtight lids.
- Put enough water to moisten the filter paper.
- Hold the petridish / germination box in slanting position in order to drain out the extra moisture.
- Record the test number and date of putting on the lid of the container or on die paper slip.
- Space the counted seeds on the moist blotter/filter paper.

- Cover the lid
- Transfer the test in the germinator maintained at the desired temperature.

**Sand Substrata(s):**

- Properly graded and sterilized sand free from impurities and toxic chemicals should be used.
- Sand should not be stored in the stores where fertilizers and chemicals are stored.
- Grade, the sand with a sieve set of 0.8 mm x 0.05 mm (mesh).
- Sand retained over 0.05 mm sieve should only be used.
- After each test, the sand should be dried and sterilized.
- If required, the sand may be washed before sterilization.
- If the sand found to be heavily contaminated or changed in colour after repeated use it should be replaced with fresh stocks.
- The pH of the sand should be within the range of 6.0- 7.5.
- The sand should also be checked if its phytotoxicity.
- Determine the, moisture holding capacity of the sand.
- Put required quantity of water to moisten the sand.
- The moisture level of the sand will vary according to the kind of seed.
- Place moist sand in plastic germination boxes. The depth of sand bed should be Approximately “2".
- Space the counted seed on the sand bed contained in the germination boxes.
- Cover the seed with moist sand layer, approximately 1/4" in thickness.
- Put the cover on the germination boxes and place them under prescribed controlled temperature conditions.

**Germination Environment**

After placing the seeds on the prescribed substrata, the test should be transferred to the controlled temperature condition maintained in the cabinet or walk-in-germinator for prescribed period, which varies according to the species (ISTA Seed Testing Rules). In the Rules for Seed Testing, two kinds of temperature conditions are provided. A single numerical indicate the constant temperature and numerical separated by a dash (-) indicate an alternating temperature. If temperatures cannot be conveniently altered over weekends or holidays, the tests must be kept at the lower temperature. The daily alternation of temperature either brought out manually by transferring the test from one germinator to another or by changing the temperature of the chamber (Automatic Seed Germinator).

**Methods to improve germination**

For many species where hard seeds occur, some special treatment is essential. This treatment may be applied prior to the commencement of the germination test or, if it is suspected that the treatment may adversely affect non-hard seeds, it should be carried out on the hard seeds remaining after the prescribed test period. The treatments are as below:

**Soaking:** Seeds with hard seed coats may germinate more readily after soaking for up to 24-48 hours in water or for Acacia spp. after plunging seeds in about three times their volume of near boiling water until it cools. The germination test is commenced immediately after soaking.

**Mechanical scarification:** Careful piercing, chipping, filing or sand papering of the seed coat may be sufficient to break the dormancy condition. Care must be taken to scarify the seed coat at a suitable part in order to avoid damaging the embryo. The best site for mechanical scarification is that part of the seed coat immediately above the tips of the cotyledons.

**Acid scarification:** Treating with in concentrated Sulphuric acid (H2S04) is effective with some species (e.g. Macroptilium sp., Brachiaria sp., Sesbania sp.). The seeds are moistened with in the acid until the seed coat becomes pitted. Digestion may be rapid or take more than one hour, but the seeds should be examined every few minutes. After digestion, seeds must be thoroughly washed in running water before the germination test is commenced. In the case of Oryza sativa scarification may be performed by soaking the seed in one normal nitric acid (HNO3) for 24 hours (after preheating at 50 °C).

**Inhibitory Substances:** Naturally occurring substances in the pericarp or seed coat, which act as inhibitors of germination may be removed by washing the seeds in running water at a temperature of 250C before the germination test is made. After washing, the seeds should be dried back at a maximum temperature of 25°C (e.g. Beta vulgaris). Germination of certain species is promoted by removing outer structures such as involucre of bristles or lemma and palea of certain Poaceae (Gramineae).

**Disinfection of the seed:** For samples of *Arachis hypoagea* and *Beta vulgaris* only, a fungicide treatment may be applied before planting the seed for germination, when the seed lot is known not to have received such a treatment. When a fungicide pretreatment is used, the name of the chemical, the percentage of active ingredients and the method of treatment shall be reported on the certificate.

**Pre chilling:** In some seeds having physiological dormancy pre chilling is required for inducing germination. Replicates for germination are placed in contact with the moist substratum and kept at a low temperature for an initial period before they are removed to the temperature as shown in (ISTA Seed Testing Rules - Table 2). Agricultural and vegetable seeds are kept at a temperature between 5°C and 10°C for an initial period up to 7 days. Tree seeds are kept it a temperature between 3°C and 5°C, for a period, varying with the species, from 7 days to 12 months. In some, cases it may be necessary to

extend the prechilling period or to rechill. The prechilling period is not included in the germination test period but both the duration and the temperature should be reported on the analysis card

**Pre-drying:** The replicates for germination should be heated at a temperature not exceeding 400C with free air circulation for a period of up to 7 days before they are placed under the prescribed germination conditions. In some cases it may be necessary to extend the pre-drying period. Both the duration and the temperature should be reported on the Analysis Certificate.

**Chemical Treatments:**

**Potassium nitrate (KNO3):** The germination substratum may be moistened with a 0.2% solution of KNO3, as indicated in (ISTA Seed Testing Rules - Table 2). The substratum is saturated at the beginning of the test but water is used for moistening it thereafter. The use of this treatment should be noted on the analysis certificate.

The procedure for preparing solutions and soaking blotters is as follows:

i) Preparation of stock KNO3, solution (2%): Place 20 Gms KNO3 crystals in 1000 ml water shake until dissolved. This must be diluted before being used to soak blotters.

ii) Preparation of 0.2% KNO3 solution for soaking blotters: Add 90 ml water to 10 ml of stock solution

iii) Procedure for soaking blotters: a. Take the blotters representing the sample and place into the prepared solution,(0.2%)-one at a time. b. Turn blotters over in one movement, but ensuring that they are still free moving in the solution. c. Remove one at a time, in order of placing in solution and place on tray.

**Gibberellic acid (GA3):** Moisten the germination substratum with 50 ppm solution of GA, which can be prepared by dissolving 500 mg of GA3 in 1000 ml of water. Place the seed for germination under prescribed temperature conditions.

**Duration of testing**

The duration of the test is determined by the time prescribed for the, final count but the chilling, periods before or during the test, which is required to break dormancy, is not included in the test period. If at the end of the prescribed test period some seeds have just started to germinate, the test may be extended for an additional period up to 7 days. A test may be terminated prior to the prescribed time when the analyst is satisfied that the maximum germination of the sample has been obtained. The time for the, first count is approximate and a deviation of 1-3 days is permitted. The First count may be delayed to permit the development of root hairs in order to be certain that root development is normal, or may be omitted. Intermediate counts may be at the discretion of the analyst to remove seedlings, which have reached a sufficient state of

development for evaluation, to prevent them becoming entangled. But the number of intermediate counts should be kept to a mini- mum to reduce the risk of damaging any seedlings that are not sufficiently developed. Seedlings may have to be removed and counted at more frequent intervals during the prescribed period of the test when a sample contains is infected with 'fungi or bacteria. Seeds that are obviously dead and decayed, and may, therefore, be a source of contamination for healthy seedlings, should be removed at each count and the number recorded.

**Evaluation of germination test**

The germination tests need to be evaluated on the expiry of the germination period, which varies according to the kind of seed. However, the seed analyst may terminate the gern1ination test on or before the final count day or extend the test beyond the period de- pending on the situation.

First and second counts are usually taken in case of Top of Paper (TP) and Between Paper (BP) media; however, a single final count is made in case of sand tests. At the first and subsequent counts, only normal and dead seeds (which are source of infection) are removed and recorded. In evaluating the, germination test, the, seedlings and seeds are categorized into normal seedlings, abnormal seedlings, dead seeds, fresh ungerminated and hard seeds. The fresh ungerminated or hard seeds and abnormal seedlings should be evaluated at the end of germination. The stage of development of the essential structures must be sufficient to permit detection of any abnormal seedlings. It may also be necessary to remove the seed coat and separate the cotyledons In order to examine the plumule in species where essential structures are still enclosed at the end of the test.

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

- Seedlings, which show the capacity for continued development into normal, plants when grown in good quality soil and under favourable conditions of water supply, temperature and light.
- Seedlings that possess all the following essential structures when tested on artificial substrata:
- A well-developed root system including a primary root-, except for those plants (e.g. ceftt1in species of Gramineae) normally producing seminal roots of which there still are at least two.
- A well-developed and intact hypocotyl without damage to the conducting tissues.

- An intact plumule with a well-developed green leaf, within or emerging through the coleoptile, or an intact epicotyl with a normal plumular bud.
- One cotyledon for seedlings of monocotyledons and two cotyledons and seedlings of dicotyledons.

Seedlings with the following slight defects provided they show vigorous and balanced development of the other essential structures:

- Seedlings of Pisum, Vicia, Phaseolus, Lupinus, Vigna, Glycine, Arachis, Gossypium, Zea and all species of Cucurbitaceae, with a damaged primary root but with several secondary roots of sufficient length and vigour to support the seedlings in soil.
- Seedlings with superficial damage or decay to the hypocotyl, epicotyl or cotyledons, which is limited in area and does not affect the conducting tissues.
- Seedlings of dicotyledons with only one cotyledon.

Seedlings of tree species having epigeal germination when the radicle is four times the length of the seed provided all structures that have developed appear normal. Seedlings which are seriously decayed by fungi or bacteria, but only when it is clearly evident that the parent seed is not source of infection and it can be determined that all the essential structures were present.

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

Seedlings with the following defects shall be classfied as abnormal:

***Damaged seedlings****;* seedlings with no cotyledons; seedlings with constrictions, splits, cracks or lesions which affect the conducting tissues of the epicotyl, hypocotyl or root; seedlings without a primary root of those species where a primary root is an essential structure, except for Pisum, Vicia, Lupinus, Vigna, Glycine, Arachis, Gossypium, Zea and all species' of Cucurbitaceae, when several vigorous secondary roots have developed to support the seedlings, in soil.

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

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

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

Special categories of abnormal seedlings The 3 main categories of abnormality, damage, deformity and decay, outlined in the previous section, can be further classified into categories as follows:

**a) Roots:**

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

**b) Hypocotyls and epicotyl:**

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

**c) Coleoptile (Gramineae):**

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

**d )Cotyledons (Dicotyledonous species):**

a. None
b. One, with evidence of damage to the shoot apex.

c. Poorly developed leaf-like cotyledon in Allium, without a definite bend, or "knee".
d. Enlarged, with short hypocotyl. e. Physiological necrosis
e. Grey in colour
f. Swollen and blackened
g. More than half the total area broken off, or covered with spots or darkened areas, or with open splits if development as a whole is out of proportion compared with that of a normal seedlings germinated at the same time.

**e) Decay:**

a. Decayed cotyledons.
b. Decayed hypocotyl.
c. Decayed epicotyl or stem.
d. Decayed plumule, or decay at point of attachment between seedlings and endosperm or discoloration of the coleoptile which has penetrated to the leaves.
e. Decayed primary root (except secondary infection by Phoma betae) or seminal roots in the Gramineae.
f. Decay or discoloration at point of attachment between cotyledons and seed- lings axis, or adjacent to the shoot apex.
g. Completely delayed seedling.

**f) Other abnormalities:**

a. Seedlings short and weak, or spindly, or watery.
b. Frost damaged seedlings with grainy coleoptile or a plumule, which is weak and spirally twisted.
c. Entirely white seedling in the Gramineae and Liliaceae.
d. Completely shattered seedling.

**Calculation and expression of result**

Results are expressed as percentage by number. Germination rate is the average number of seeds that germinate over the five-day and 10-day time period.

Germination (%) = Number seeds germinated x 100 Number seeds on tray

When four l00-seed replicates of a test are within the maximum tolerated range the average represents the percentage germination to be reported on the Analysis Certificate. The average percentage is calculated to the nearest whole number. The total % of all the category of seeds (normal, abnormal. dead hard, fresh un germinated) should be 100.

**Retesting**

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

- When dormancy is suspected (fresh ungerminated seeds).
- When the result may not be reliable because of phytotoxicity or spread of fungi or Bacteria.
- When there is difficulty in deciding the correct evaluation of a number of seedlings.
- When there is evidence of errors in test conditions, seedling evaluation or counting.
- When the range for the 100-seed replicates exceeds the maximum tolerated range

**Reporting of result**

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

- Kind of variety
- Date of testing
- Duration of test
- Percentage of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds.

If the result for any of these categories is found to be nil, it shall be entered as 0
The following additional information shall also be reported:

- In all cases
- Substrate and temperature used.
- Any special treatment or method used for promoting germination.
- The germination percentage obtained within the prescribed time, if the germination period has been extended beyond the period indicated.
- The second result obtained when duplicate tests are indicated in Table SA.

Upon request

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

# DETERMINATION OF SEED MOISTURE CONTENT

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The seed moisture content (mc) is the amount of water in the seed. It is usually expressed as a percentage on wet weight basis in any seed-testing laboratory. The seed moisture content is the most vital parameter, which influence the seed quality and storage life of the seed. Seed moisture content is closely associated with several aspects of physiological seed quality. For example, it is related to seed maturity, optimum harvest time, mechanical damage, economics of artificial seed drying, seed longevity and insect & pathogen infestation.

**Objective**

The objective is to determine the moisture content of seed by methods suitable for routine use.

**Definition**

The moisture content of seed sample is the loss in weight when it is dried in accordance with ISTA rules. It is expressed as a percentage of the weight of the original sample.

**Principle**

The methods prescribed are designed to reduce oxidation, decomposition or the loss of other volatile substances while ensuring the removal of as much moisture as possible.

**Seed ageing and loss of seed germination:** Seed ageing and loss of germination cannot be stopped, but can be minimized by proper seed storage conditions. The important environmental *factors influencing germination loss are relative humidity* (RH) governs the seed moisture, and *temperature,* higher are these; the more rapid is the seed deterioration. The effects of seed moisture and temperatures are summarized succinctly in *Harrington's rules,* as under:

1. For every decrease of 1 per cent in seed moisture content the life of seed is doubled.
2. For every decrease of 5$^{o}$C in storage temperature the life of the seed is doubled.

Rule 1 hold's good, when the seed moisture content is between 14 and 5 per cent. When these two simple rules are applied, the effects are geometric.

**Determination of seed moisture content**

As seed moisture and its management influences so many physiological seed quality parameters essential to seed quality. Seed moisture measurement appropriate to the purpose is needed in commerce and research.

The optimum method for moisture testing depends upon:

a) Chemical composition of seed
b) Seed structure
c) Moisture content level
d) Degree of accuracy and precision required
e) Constraints of time
f) Technical expertise and cost

The ideal could be that is adopted to all seeds, measures moisture content from 0 to 100 per cent, reproducible, require less training and low in cost. It is impossible to combine all these. However, in order to measure the moisture content of seeds, methods can be broadly grouped in two categories:

1. Direct method
2. Indirect method

**Direct method**

Under this category, the seed moisture content is measured directly by loss or gain in seed weight.

These are:

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

**Indirect method**

These are no so accurate; estimation is approximate, but convenient and quick in use. These are frequently used at seed processing plants. These measure other physical parameters like electrical conductivity or electrical resistance of the moisture present in the seed. Values are measured with the help of seed moisture meters, and these values are transformed into seed moisture content with the help of calibration charts, for each species, against standard air-oven method or basic reference method.

Above all Karl-Fisher's method has been considered as the most accurate and the basic reference method for standardizing other methods of seed moisture determination. The constant temperature oven drying method is the only practical method, approved by International Seed Testing Association (ISTA) and other organization to be used for routine seed moisture determination in a seed-testing laboratory.

**Constant temperature oven drying method**

The constant temperature oven drying method is broadly grouped into two categories:

I. Low Constant Temperature Oven Method
II. High Constant Temperature Oven Method

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

**High Constant Temperature Oven Method:** The procedure is the same as above except that the oven is maintained at a temperature of 130°-133°C. The sample is dried to a period of four hours for *Zea mays*, two hours for other cereals and one hour for other species (see also Table 2). In this method, there is no special requirement pertaining to the relative humidity of the ambient air in the laboratory during moisture determination.

**Essential equipments and supplies**

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

Fig.1.Hot air oven

Fig.2. Dessicator

Fig. 3. Electric balance

Fig. 4. Grinding mill


Fig. 5. Sieves

**Period of seed drying**

The prescribed period of seed drying shall be 17± 1 hrs at 103°C under low constant and 1to 4 hrs at 130°-133°C under high constant temperatures. Maize seed be dried for 4 hrs, cereals and/or other millets for 2 hrs and the remaining species for 1 hr. Seeds rich in oil content or with volatile substances be dried for 17± 1 hrs under low constant temperature. Seed drying period begins from the time oven returns to maintain the desired temperatures.

**Sample size**

The ISTA rules recommend that two replicates, each with 4 gm of seed be used for determination of seed moisture content. This seed sample weight may be modified to 0.2 to 0.5 gm per replicate, with precise weighing, for use in seed genebanks, to avoid unnecessary depletion of precious biological resources.

**Procedure**

i. Seed moisture determination be carried out in duplicate on two independently drawn working samples
ii. Weigh each bottle with an accuracy of 1 mg or 0.1 mg.
iii. First weigh the empty bottle/container with its cover
iv. Grind the seed material (if need be, in seed species listed in Table 3)), evenly using any grinder/grinding mill that does not cause heating and/or loss of moisture content.
v. Mix thoroughly the submitted sample, using spoon, and transfer small portions (4 to 5 gm) of seed samples directly into weighing bottles/containers, by even distribution on bottom of the containers.
vi. After weighing, remove the cover or lid of the weighing bottles/containers.
vii. Place the weighing bottles/containers in an oven, already heated to or maintaining the desired temperature, for the recommended period.
viii. At the end of seed drying period, weighing bottles/containers be closed with its lid/ cover.
ix. Transfer the weighing bottles/containers to the desiccators having silica gel(self indicating –blue), to cool down for 40 to 45 min.
x. Weigh again the cooled weighing bottles/containers.
xi. Calculate the seed moisture content.

**Calculation of results**

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

$$\text{Percentage seed moisture content} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where

$M_1$ = Weight of the weighing bottle/container with cover in gm

$M_2$ = Weight of the weighing bottle/container with cover and seeds before drying

$M_3$ = Weight of the weighing bottle/container with cover and seeds after drying

{Note: The seed moisture determination must be done in two replicates, with precise weighing (ie up to three decimal places) using lightweight weighing bottles/containers.}

**If the seed is pre-dried or dried in two steps:** The seed moisture content is calculated from the results obtained in the first (pre-dried) and second stages of seed drying, using the following formula, and expressed as percentage, as under:

$$\text{Percentage seed moisture content (mc)} = \frac{(S_1+S_2) - (S_1 \times S_2)}{100}$$

*Where,*

$S_1$ = is the moisture loss in the first stage, and

$S_2$ = is the moisture loss in the second stage

**Moisture meters**

**Universal (OSAW) digital moisture meters:**

The principle involved in these moisture meters is that wet grains are good conductors while dry grains are less conductors of electricity. So, the moisture content is directly proportional to the electrical conductivity of the seed.

It consists of a compression unit to compress the sample to pre -determined thickness. The thickness setting is very easily read on a vertical and circular scale. The seed material on test is taken in a test cup and is compressed. Then press the push type switch till the reading comes in the display. Here no temperature reading and correlated dial are required. The computer version of digital moisture meter automatically compensate for temperature corrections.

**Use of tolerances**

Result is the arithmetic mean of the duplicate determination of seed moisture content, for a given seed sample. The maximal difference of 0.2% is recommended between two replicates, for crop seed species under ISTA rules. If the difference between two replicates exceeds 0.2%, the seed moisture determination in duplicate be repeated.

As it is very difficult, rather impossible, to meet the replicated difference of seed moisture up to 0.2% in tree or shrub species, a maximal limit of 0.3 to 2.5% is recommended between two replicates for seed moisture in tree or shrub species under ISTA rules.

**Reporting of results**

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

**Table 1:** Species for which the low constant temperature (103°C) oven method be used

| | |
|---|---|
| *Allium* spp | *Linum ustatissimum* |
| *Arachis hypogea* | *Raphanus sativus* |
| *Brassica* spp | *Ricinus communis* |
| *Camelina sativa* | *Sesamum indicum* |
| *Capsicum* spp | *Sesamum orientale* |
| *Glycine max* | *Sinapsis* spp |
| *Gossypium* spp | *Solanum melongene* |

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

| | | | |
|---|---|---|---|
| *Agrostis spp* | *Citrullus lanatus* | *Lolium spp* | *Phaseolus spp* |
| *Alopecurus pratensis* | *Cucumis spp* | *Lotus spp* | *Phelum spp* |
| *Anethum graveolens* | *Cucurbita spp* | *Lupinus spp* | *Pisum sativum (all varieties)* |
| *Anthoxanthum odoratum* | *Cuminum cyminum* | *Lycopersicon lycopersicum* | *Poa spp* |
| *Anthriscus spp* | *Cynodon dactylon* | *Medicago spp* | *Scorzonera hispanica* |
| *Apium graveolens* | *Cynosurus cristatus* | *Melilotus spp* | *Secale cereale* |
| *Arrhenatherum spp* | *Dactylis glomerata* | *Nicotiana tabacum* | *Sorghum spp* |
| *Asparagus officinalis* | *Daucus carota* | *Onobrychis vicifolia* | *Spinacea oleracea* |
| *Avena spp* | *Deschampsia spp* | *Ornithopus sativus* | *Trifolium spp* |
| *Beta vulgaris (all varieties)* | *Fagopyrum esculentum* | *Oryza sativa* | *Trisetum flavescens* |
| *Bromus spp* | *Festuca spp* | *Panicum spp* | *Triticum spp* |
| *Cannabis sativa* | *Holcus lanatus* | *Papaver somniferum* | *Valerianella locusta* |
| *Carum carvi* | *Hordeum vulgare (all varieties)* | *Paspalum spp* | *Vicia spp* |
| *Chloris gayana* | *Lactuca sativa* | *Pastinaca* | *Zea mays* |

| | | | |
|---|---|---|---|
| | | *sativa* | |
| *Cicer arietinum* | *Lathyrus spp* | *Petroelimum crispum* | |
| *Cichorium spp* | *Lepidium sativum* | *Phalaris spp* | |

**Table 3:** Species for which grinding is obligatory

| | | | |
|---|---|---|---|
| *Amorpha fruticosa* | *Fagopyron esculantum* | *Lupinus* spp | *Secale cereale* |
| *Arachis hypogaea* | *Glycine max* | *Oryza sativa* | *Sorghum* spp |
| *Avena* spp | *Gossypium* spp | *Phaseolus* spp | *Triticum* spp |
| *Cicer arietinum* | *Hordeum vulgare* | *Pisum sativum* | *Vicia* spp |
| *Citrullus lanatus* | *Lathyrus* spp | *Ricinus communis* | *Zea mays* |

# SEED VIABILITY AND VIGOUR TESTING: PRINCIPLES AND METHODS

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## SEED VIABILITY TESTING

Germination test is the standard test to judge the potential of a given seed lot for emergence under field conditions. It takes from days to weeks, and in some cases even months to complete. Therefore, for quick assessment of seed viability Tetrazolium test is commonly used to furnish quick estimate of seed germinability based on its respiratory activity. It is also known as the quick viability test, TZ test and Topographic Tetrazolium Chloride Test (TTC).

Seed viability indicates that a seed contains structures and substances, enzyme system which give it the capacity to germinate under favourable condition in the absence of dormancy. There have been approaches since long for biochemical methods for assessing viability of seeds (planting value) without standard germination test. Lakon (1942) for the first time reported that tetrazolium salt can be used to assess the viability of seeds. Since then the method has been used in a number of crops. TZ test is suitability modified and improved to assess the seed quality in terms of this viability in different crops.

### Objectives

The objective of biochemical tests are:

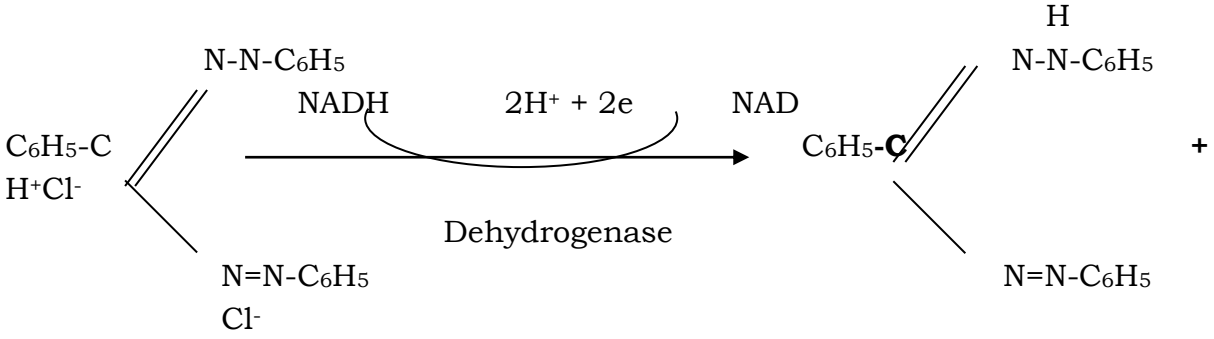
a. To make quick estimate of the viability of the seed sample in general and those showing dormancy in particular.
b. In the case of particular sample which at the end of a germination test reveal a high percentage of dormant seeds, to determine the viability of individual dormant seeds or the viability of a working sample.

### Principle

The test is based on the principle that the living tissues, which respire, can reduce the colourless solution of 2,3,5 Triphenyl Tetrazolium Chloride or Bromide into a non-diffusable red coloured compound, known as formazan. The reduction of the chemical is achieved in the seed by the action of a group of enzymes known as dehydrogenases. These enzymes are involved in H-transfer during respiratory activity of biological system. Since the reaction takes place within the respiring (living) cells and the formazan is non-diffusable, a clear topography of living (red) and non-living areas (white) within the seed is being developed by using proper procedure. In addition to completely stained viable seeds and completely unstained non-viable seeds, particularly stained seeds may developed varying proportions of necrotic tissues. The positions and size of necrotic areas determine whether seeds are classified as viable or non-viable.

**Chemical Reaction**

The colourless solution of 2,3,5-tri-phenyl tetrazolium chloride/bromide (a cream or light yellow soluble powder) in the presence of certain dehydrogenase enzymes (released during respiration by living tissue) changes into an insoluble red (stained) formazon.




2,3,5-Triphenyl Tetrazolium Chloride (Colourless)

Formazon (Red, non-diffusible)

**Advantages of TZ test**

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

**Disadvantages of TZ test**

- It is difficult to distinguish between normal and abnormal seedlings.
- It does not differentiate between dormant and nondormant seeds.
- Since, the TZ test does not involve germination, micro-organisms harmful to germinating seedlings are not detected.

**Accuracy of the Tetrazolium Test depends on**

- A sound knowledge of seed and seedling structures.
- An understanding of the mechanism of the reaction leading to the staining of the seed.
- Ability to interpret the staining pattern by critically examining the seed turbid topography and by keeping other visible seed qualities such as tissues turbidity, abnormal or missing structures etc. in view and
- Experience gained through interest and natural skill.

**Essential Seed Structures for TZ Test Evaluation**

Seeds can be grouped into monocots, dicots and polycots on the basis of the presence of one, two or more than two cotyledons. Embryo structures or

structure primordial are, relatively similar for embryos within each group though they may differ in shape, size and differentiation/development of embryo structures. The critical features of the seeds of these groups are described below.

a. **Monocotyledons:** Seeds of gramineae family are classical example of this group. The most critical features are the growing root and shoot tips and the scutellum. The root region comprises mainly of the radicle, which may be the only root primordial in some cases e.g.,. small seeded grasses or it may have several seminal root primordial besides the radicle e.g., wheat, maize, barley etc. The radicle lies below the mesocotyl region covered under a sheath termed as coleorrhiza. The shoot region or the plumule consist of the shoot meristem along with one or more leaf primordial near the base, enclosed within the coleoptile. Msocotyl separates plumule from the point of attachment to the scutellum.
b. **Dicotyledons**: In the dicot seeds with a low degree of differentiation the embryo is embedded in nutritive tissue and consists of a radicle and two cotyledons. In the seeds with a higher degree of differentiation, embryo occupies a larger portion of the seed. The radicle is well recognizable part of the embryo, while the shoot meristem with the primary leaf primordial generally lies compressed between the two cotyledons.
c. **Polycotyledons**: Polycotyledons are not very common. This type of seed is found in pinaceae and other conifers. The embryo is not very well differentiated except for a radicle and more than two cotyledons. Living nutritive storage tissues encircle the embryo, whereas, the shoot meristem is encircled by the base of the cotyledons.

**TZ test methodology**

The regular germination tests are easy to do and interpret. The tetrazolium tests and the interpretation of results are more difficult. For a quick estimate of seed viability, the following should be considered:

**Equipments:** Incubator, Cabinet germinator, Knife, Petri dishes, Razor, Scalpel, etc.

**Reagent:**

- One per cent solution of 2,3,5 triphenyl tetrazolium chloride or bromide of pH 6.5-7.5 (TZ): An aqueous solution of 2,3,5-triphenyl tetrazolium chloride or bromide within a pH range of 6.5-7.5 is used. The concentration normally used is 1.0 per cent. Sometimes lower or higher percentage are more appropriate. To get 1% of TZ solution, dissolve 1 g of TZ salt in 100 ml of buffer solution.
- Potassium dihydrogen phosphate and disodium hydrogen phosphate.
  - ❖ Solution 1 – dissolve 9.078 g $KH_2PO_4$ in 1000 ml water

- Solution 2 – dissolve 9.472 g $Na_2HPO_4$ in 1000 ml water or dissolve 11.876 $Na_2HPO_4.2H_2O$ in 1000 ml water.

Mix two parts of solution 1 with three parts of solution 2 to get a buffer solution of neutral pH.

The suggested methods for TZ test methods for common agricultural crop seeds are as follows:

| Kind of seed | Preparation | Concentration of Solution (%) | Staining Time at 35$^0$C (hr) |
|---|---|---|---|
| Alfalfa | No preparation | 1.0 | 6-7 |
| Barley | Bisect longitudinally | 0.1 | 2-3 |
| Bean | No Preparation | 1.0 | 3-4 |
| Blue grass | Bisect Laterally or pierce | 1.0 | 6-8 |
| Clovers | No preparation | 1.0 | 6-7 |
| Corn | Bisect longitudinally | 0.1 | ½ -1 |
| Cotton | Remove seed coat | 1.0 | 3-4 |
| Fescue | Bisect longitudinally | 0.1 | 2-3 |
| Oats | Bisect longitudinally | 0.1 | ½ -1 |
| Peanut | Remove seed coat | 1.0 | 3-4 |
| Peas, Field | No preparation | 1.0 | 3-4 |
| Rape | Remove seed coat | 1.0 | 3-4 |
| Rice | Bisect longitudinally | 0.1 | 3-4 |
| Rye | Bisect longitudinally | 0.1 | ½-1 |
| Sorghum | Bisect longitudinally | 0.1 | ½-1 |
| Soybean | No preparation | 1.0 | 3-4 |
| Sunflower | Remove seed coat | 1.0 | 3-4 |
| Wheat | Bisect longitudinally | 0.1 | ½-1s |

**Working Sample**

At set of 100 seeds be tested in replicates of 50 each or less. For an accurate assessment, the test is conducted in 4 x 10 seeds. The seeds should be randomly drawn from the pure seed component and counted in replicates before conditioning.

**Temperature of Staining**

Temperature influences the staining reaction. Staining will take place twice as fast at 30$^0$ C as that of 20$^0$ C, and twice as fast at 40$^0$ C as that of 30$^0$ C. Temperatures between 20$^0$ C and 40$^0$ C have no effect on accuracy of tetrazolium test, but staining proceeds faster at the higher temperature. The test can be performed satisfactorily at room temperature.

**Pre-conditioning**

In order to ensure the contact of the tetrazolium solution with the embryo, some conditioning and preparatory steps may be essential. In addition to moistening, most kind of seeds require some preparatory steps before staining, to

- assure the adequate penetration of the staining solution into the seed
- accelerate the rate of staining
- facilitate the evaluation.

These depend on the type of the seed, its permeability and thickness of the seed coat, location of the embryo etc. It is done to

- allow complete hydration of all the tissues
- prevent damages to cotyledons and embryo axes, during cutting of seeds
- initiate and activate the germination process
- have proper penetration of tetrazolium solution.

Some seeds can directly be placed in water, whereas, others must be moistened slowly. Slow moistening is generally practiced for large seeded legumes or for the seed samples, which are dry enough, brittle or are aged to avoid tissues damage (due to rapid intake of water). Seeds are conditioned either by placing the seed on top of or in between the moist blotter or paper towels. Thus, depending upon the kind of the seed, time available, degree of accuracy desired and the experience of the analyst, one the following methods may be adopted:

1. No moistening or preparation (small seeded legumes with soft coats)
2. Slow moistening without any preparation (large seeded with soft coats)
3. Piercing, puncturing or cutting of the seed coat (small seeded grasses)
4. Cutting the seeds longitudinally through the midsection of the embryo and through part of the endosperm, leaving the two halves attached at the base or slitting the seed completely, keeping only half for the staining, discard the other
5. Cutting the seeds longitudinally slightly off-center to avoid cutting into the embryo
6. Removing the seed coat (with forceps/needle/razor blade etc.) with minimum injury to the tissues. Sometimes, a thin membrane adheres to the cotyledons even after removal of the seed coat (*e.g.,* sunflower, cotton), this be removed by a sliding motion, after an additional 30 min of soaking (dicots with hard seed coats).

**Staining**

Seeds can be stained in watch glass, petri dishes or beakers. Sufficient solution should be used to cover the seeds and to allow its absorption. As a rule of thumb, a concentrated (1.0%) solution can be use for legumes, cotton and grasses that are not bisected through the embryo and a dilute (0.25% or 0.50%) solution for grasses and cereals that are bisected through the embryo.

In general, seeds are placed in the solution and held at $30^0$ C for complete colouration. After a period in the solution, the length of which varies according to the condition of the seed, species, temperature and concentration of the solution, seeds are rinsed 2-3 times in water and then evaluated. During evaluation, the seeds should be left in a little water to prevent these from drying. If seeds are not to be evaluated immediately, the seeds in a little water should be kept in the refrigerator ($5^o$ to $10^o$ C). Whenever required, 2-3 drops of lactophenol (clearing solution) be added to 100 seeds of small seeded grass, after completely removing the tetrazolium solution. Small seeds are examined under a stereomicroscope, while large seeds may be examined with magnifying lenses. Seed coats of legumes must usually be removed before examination.

**Evaluation of sample**

The main purpose of the tetrazolium test is to distinguish viable and non-viable seeds. The sample is ready for evaluation when it is stained. Observe the staining pattern and calculate the percentage of viable seed. Each seed is examined and evaluated as viable or non-viable on the basis of the staining patterns and tissue soundness revealed. Viable seeds are those that show the potential to produce normal seedlings. Such seeds stain completely, or if only partly stained, the staining patterns indicate that the essential structures are viable.

Non-viable seeds are those that do not meet these requirements and in addition include seeds that reveal uncharacteristic colouring and/or placcid essential structures. Seeds with obviously abnormal development of the embryo or other essential structures shall be regarded as non-viable whether stained or not. Rudimentary embryos of coniferous seeds are non-viable.

In order to evaluate seeds properly, it is necessary to expose the embryo and other essential structures. Appropriate light and magnification are indispensable for proper examination. Most seeds contain essential and non-essential tissues. Essential structures are the meristems and all structures recognized as necessary for the development of normal seedlings.

**Interpretation of TZ Test Results**

The Seed Technology laboratory, Mississippi State University and ISTA developed detailed staining patterns of some of the major crop species for interpreting of the TZ test result. Most of the other crops can be evaluated taking one or the other of these staining patterns as the guide. However, for some crops, it may be necessary to first standardize the staining.

To interpret the staining pattern of seed correctly, as viable or non-viable (dead), the seed analyst must understand correctly the essential structures of the embryo and their role in the growth of seedling. After differentiating viable seeds from the non-viable ones, the viable seed group can be reclassified into several vigour groups on the basis of intensity of stain and staining pattern of

different seed parts. The simplest classification is to divide viable seeds lot having larger number of sound seeds considered to be more vigorous.

The term *stained* refers to viable (functional) tissues as established by progress of staining characteristics of stain, and/or appearance and turgidly moist stained or non-stained tissues. The term *necrosis* refers to non-viable (non-functional) tissues as determined by abnormal staining and lack of turgidly moist stained or non-stained tissues. Evaluation codes provide only partial guidance for deterioration of seed viability. The projected influence upon normal seedling development of fractures, abnormalities, missing tissues, etc. must also be considered. A few seeds, fully stained (with tetrazolium chloride), do not germinate; require comparison against standard germination tests.

If conducted following all guidelines, TZ test results are in close agreement with the germination percentage. However, discrepancies between the two results might arise due to several factors:

1. Sample difference
2. Improper germination testing
3. Improper TZ testing
4. Seed dormancy
5. Presence of hard seeds
6. Presence of seed-borne organism.
7. Chemical injury (such as fumigation injury and excess treatment with mercurial fungicides etc., which may not inhibit the TZ staining but could affect normal germination).

**Calculations**

The results are reported as percentage based on colour intensity and topography in relation to total seed tested.

| **Seed Numbers** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **.** | **.** | **.** | **n** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMBRYO** | | | | | | | | | | | | | | |
| Complete stained | | | | | | | | | | | | | | |
| Not staining | | | | | | | | | | | | | | |
| Necrosis* | | | | | | | | | | | | | | |
| **COTYLEDON** | | | | | | | | | | | | | | |
| Complete stained | | | | | | | | | | | | | | |
| Not staining | | | | | | | | | | | | | | |
| Necrosis* | | | | | | | | | | | | | | |
| **COLOUR** | | | | | | | | | | | | | | |
| Red | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pink | | | | | | | | | | | | | | |
| Fractured | | | | | | | | | | | | | | |

*Decision on the basis of expression in specific structure

**Practical Consideration**

The TZ test is reliable and widely utilized. TZ test may be used to make a rapid assessment of seed viability, when seeds have to be sown shortly after harvest or in cases, where a very quick estimate of germination potential is required. The test is very useful in processing, handling, storing and marketing large quantities of seed in a short time, testing dormant seed lots or seed with deep dormancy, vigour rating of the seed lots or in seeds showing slow germination, supplementing germination test result and diagnosing the cause of seed deterioration. It can also be used, to determine the viability of individual seeds at the end of a germination test, where dormancy is suspected and to solve problems encountered in a germination test, *e.g,.* when reasons for abnormal are not clear, treatment with pesticides is suspected, etc.

If the result is to be reported on an International Seed Testing Association (ISTA) International Seed Analysis Certificate, the test must be carried out strictly in accordance with the methods described in the ISTA Rules.

**SEED VIGOUR TESTING**

High seed vigour is required for germination of seed lots of cultivated species, especially under sub-optimal conditions, i.e., temperature and water-deficit. Seed germination may differ substantially among different seed lots of the same species in field emergence when sown at the same time in the same field, or differ in performance after storage in the same environment. The basic question come in mind is that when looking at the germination data for various seed lots are: "Were the germination results are wrong?" and "Why the difference in performance?" The answer to the first question is no, the germination results were correct. The answer to the second question is that it has become apparent that the germination test is not sensitive enough to indicate suitable but significant quality differences among high germinating seed lots. These differences are caused by a component of seed quality, i.e. seed vigour.

It is essential, however, that seed analysts and consumers understand that vigour tests are not designed to predict the exact number of seedlings that will emerge and survive in the field, although many of the vigour test results are well related to field emergence. One of the primary purposes of vigour test is to indicate whether or not trouble may be expected from a high germinating seed lot if the lot is placed under adverse environmental conditions in the field, storage or during transportation.

Several definitions have been offered to explain seed vigour. The ISTA congress in 1977 adopted the definition of seed vigour as "the sum total of those properties of the seed which determine the level of activity and performance of

the seed or seed lot during germination and seedling emergence". Although differences in physiological attributes of seed lots can be demonstrated in the laboratory, however it is recommended that the term should be used to describe the performance of seeds when sown under the field conditions (Perry, 1984). Since, seed germination test is conducted in an optimum condition which is specific to species. Thus the test conducted with a seed lot in the field may differ with the test conducted under laboratory conditions. As under field conditions emerging seeding may suffers from the sub-optimal conditions, such as, various biotic and abiotic stresses. In many cases seed lots having similar laboratory germinations may give widely differing field emergence values. Similarly, two seed lots having the same germination percentage in the laboratory may age differently when stored under ambient condition. These two situations indicate the incompleteness of germination test in assessing the performance of a seed lot in the field or storage. This offers scope and possibility to determine vigour of a seed lot so that its field and storage performance can be assessed. Seed vigour is still a concept rather that a specific property of a seed or seed lot. Several factors, i.e, genetic constitution, environment and nutrition of mother plant, maturity at harvest, seed weight and size, mechanical integrity, deterioration and ageing and pathogens are known to influence seed vigour (Perry, 1984). Two criteria have been employed by the ISTA seed vigour committee to evaluate, the performance of seed vigour test methods for different crops: (i) Reproducibility of vigour method (ii) The relationship between vigour test results and seedling emergence in field soil. There is no universally accepted vigour test for all kinds of seeds, therefore following tests are listed to be performed on different crops.

In one of the first attempt to conceptualize seed vigour, Isley (1957) defined it as *"the sum total of all seed attributes which favor stand establishment under unfavorable field conditions*". Similarly International Seed Testing Association (ISTA) defines seed vigour as "*the sum total of those properties of the seed which determine the potential level of performance and activity of a non-dormant seed or seedlots during germination and seedling emergence*". Seeds which perform well are termed as 'High vigour' seeds (Perry, 1972).

**Factors Affecting Seed Vigour**

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

periods of low temperature stress, genetic damage and the accumulation of toxic metabolites are all associated detrimental factors.

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

**Objectives of Vigour Tests**

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

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

- To provide a more sensitive index of seed quality than the germination test.
- To provide a consistent ranking of seed lots in terms of their potential performance.
- To be objective, rapid, simple and economically practical.
- To be reproducible and repeatable.

**Seed Vigour Testing Methods**

As early as 1876, Friedrich Nobbe described vigouras "Triebkraft" a German word meaning "Driving Force". Scientists have long searched for a way to describe the differences amongst seed lots with comparable germination. Numerous tests, theories and methodologies have been used to test for, and describe seed vigour. Over the years, many seed vigour tests have been developed and evaluated. A simple classification is given in Table 1. Sometimes a combination of tests is used on a seed lot to read its planting potential more accurately.

**Table 1:** Classification of seed vigour tests

| Seed appearance | Performance Based | Stress Based | Biochemical based |
|---|---|---|---|
| • Seed Size | • First Count | • Cool germination test | • GADA test |
| • Seed lustre | • Speed of germination | • Cold germination test | • TZ Test |

- Seed density
- Seedling growth rate and dry weight
- Brick gravel test
- Paper piercing test
- Accelerated ageing test
- Controlled deterioration test
- Respiratory quotient [R.Q.]
- Membrane integrity test

## I. PERFORMANCE BASED TESTS

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

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

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

$$\text{Lot A} = \frac{\text{No. of Seedling removed}}{\text{Day after planting}}$$

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

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

$$\text{Lot B} = \frac{\text{No. of Seedling removed}}{\text{Day after planting}}$$

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

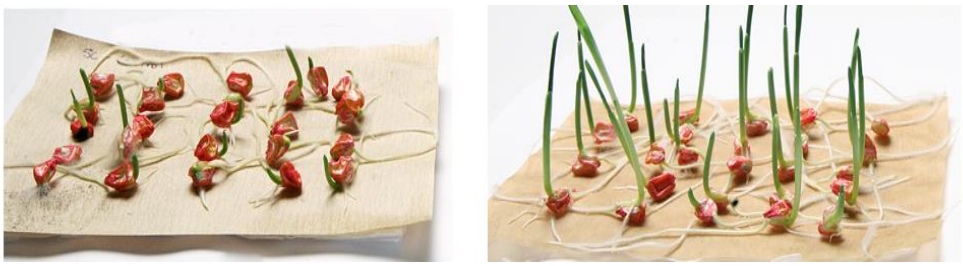
$$= 0+0+4+6+9+1 = 20$$

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

**High and low vigour lots of Wheat**

***C. Seedling Growth Rate and Seedling Dry Weight***

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



**Low and high vigour lots of maize**

**II. Stress based tests**

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

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

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

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

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

**Cold test in maize**

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

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

the physical stresses involved, and the test thus provides a method to screen seed lots for vigour.

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

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

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

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

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

### III. Biochemical Tests

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

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

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

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

Medium Vigour : Embryo completely stained or embryonic axis stained in dicots. Extremities may be unstained. Some over stained/less firm areas exist.

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

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

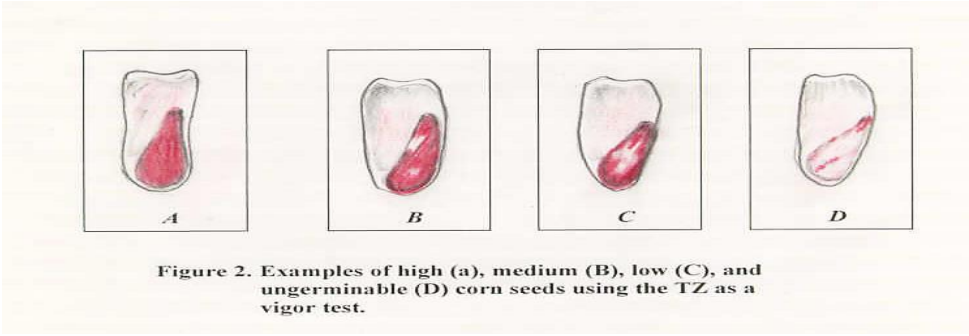



Figure 2. Examples of high (a), medium (B), low (C), and ungerminable (D) corn seeds using the TZ as a vigor test.

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

***D. Membrane Integrity Test:***Also called as Conductivity Test, provides a measurement of electrolyte leakage from tissues and was first recognized by Hibbard and Miller (1928). Seed deterioration is associated with loss of membrane integrity. When deteriorated seeds

are soaked in water they lose more electrolytes (amino acids, sugars, organic acids etc.) which increase the conductivity of water. A high conductivity of this solution denotes low vigour. In the standard conductivity test, results are expressed as mean conductivity per gram of seed weight obtained after soaking 25-50 seeds for 24 hours. This test has proved highly successful on garden pea but good results have also been resulted have also been obtained with large seeded legumes and grains. This test has been recommended by ISTA Seed Vigour Testing Committee for garden peas.

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

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

**Among these tests ISTA has approved following vigour tests**

- Accelerated ageing test for *Glycine max*
- Conductuctivity test for *Pisum sativum*
- Controlled deterioration test for *Brassica spp.*
- Radical emergence test for *Zea mays*

**Advantages of seed vigour tests**

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

**Limitations of seed vigour tests**

1. A precise referee testing method among seed laboratories is needed to arrive at a common vigour test methodology.
2. Seed vigour tests do not predict percentage field emergence.
3. Values obtained from seed vigour tests are relatively values, not absolute values of vigour.
4. Comparison of the results of different tests is difficult because results are expressed in different units.

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

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# SEED HEALTH TESTING: MOLECULAR APPROACHES

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## Introduction

Seed is a small embryonic plant enclosed in a covering called seed coat. Seed is the product of ripened ovule of gymnosperms and angiosperm plants which occurs after fertilization and some growth within the mother plant. Seed is the basic unit of production for the world's food crop. In recent years seed has become an international commodity used to exchange germplasm around the world. However, seed is also an efficient source of introducing plant pathogens into a new area as well as providing a means of their survival from one cropping season to another. So, seed health testing is thus routinely carried out in most countries for domestic seed certification, quality assessment and plant quarantine. Seed health testing is an integral for all seed companies in disease risk management.

Seed health is a well organized factor in the modern agricultural science for desired plant population and good harvest. Seed borne pathogens are a continuing problem and may even be responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas. Seed borne pathogens present a serious threat to seedling establishment. In today's global economy, seed accounts more than ever for the movement of plant pathogens across vast distances, natural barriers and political borders. The quality of planted seeds has a critical influence on the ability of crops to become established and to realize their full potential of yield and value.

## Seed health testing

Seed health is a measure of freedom of seeds from pathogens. The presence or absence of seed borne pathogens can be confirmed through the use of seed health testing. The term "seed health" includes the incidence of fungi, bacteria, viruses and animal pests such as nematodes and insects in the seed lot. The test used depends on the organism being tested for and the purpose of the test quality assurance or phytosanitary purposes when seed is exported. Seed testing is necessary for a number of reasons: to determine the quality of the seed based on a number of seed quality attributes; to provide a basis for price and consumer discrimination among seed lots and seed sources; to determine the source of a seed problem, thereby facilitating any corrective measure(s) that may be required; and to fulfil legal and regulatory requirements for certified seed classes and allow for seed movement across international boundaries. There are six main requirements for selection of seed health tests methods namely,

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

**Molecular methods in seed health testing:**

Molecular methods are able to provide precise, reliable and reproducible results rapidly. They facilitate early disease management decisions. Biochemical, immunological and nucleic acid based methods are having distinct advantages. These are useful in the identification of obligate parasites. The availability of antisera, primers and commercial kits are useful for on-site detection during field surveys for assessing the distribution of existing pathogen as well as new/introduced pathogens through seeds/material. Among the tools available for pathogen detection, nucleic acid based techniques are most accurate and the most important aspect is that these are time saving. Some of the molecular methods used in seed health technology are listed below:

**1. Restriction fragment length polymorphism (RFLP)**

A small DNA segment from a known bacterium, pathogenic to the host plant in question, is used as a probe. The DNA from both the known (as positive control) and suspected bacterial pathogen (isolated from infected plant samples) are digested with the same restriction enzyme(s). The samples of both control as well as unknown digested bacterial DNA are placed side by side in an agarose gel, and are then separated by size using electrophoresis. The double-stranded DNA fragments are then chemically denatured into single-strands and are transferred to a nylon membrane which fixes their positions and maintains them as single-stranded DNA. The nylon membrane is washed with a solution containing many copies of a radioactive DNA probe. A dark band will appear at each location where the probe hybridized to complementary DNA when nylon membrane exposed to x-ray film.

**2. Random Amplified Polymorphic DNA (RAPD)**

RAPD markers will be useful for determining polymorphism in phytopathogenic bacteria. Amplicons obtained through RAPD markers could be exploited as sequence characterized amplified regions (SCAR) marker after sequencing and designing primer specific to that region of DNA

**3. Amplified Fragment Length Polymorphism (AFLP)**

AFLP involves amplification of specific region of genomic DNA through PCR (using a single primer) followed by the cleaving of the amplified fragments using restriction endonuclease. The technique is a combination of RFLP and PCR techniques and is extremely useful in detection of polymorphism between closely related bacterial pathogens.

**4. Polymerase chain reaction (PCR)**

Polymerase chain reaction (PCR) is the *in vitro,* primer directed, enzymatic amplification of nucleic acids. For PCR, primers (small oligonucleotide probes) designed to anneal to specific DNA sequences in the target organisms chromosomal DNA or RNA, hybridize with and direct amplification of millions of copies of the target sequence. This amplified DNA can be visualized after electrophoresis in ethidium bromide stained agarose gels. To unlock the invaluable potential of PCR for detecting seed borne pathogens, several modifications have been developed. Some of the variants of PCR are:

i) ***Bio PCR:*** Bio-PCR consists of the preventive growth of target pathogens on selective media and their selective increase, relative to non-target microorganisms, followed by DNA extraction and amplification by PCR. Successfully used for detection of *Pseudomonas syringae pv. phaseolicola* in bean seed extracts.

ii) ***Immunomagnetic separation and PCR (IMS-PCR):*** In IMS-PCR, small magnetic beads, coated with antibodies for a specific microorganism, are able to selectively bind target cells from suspensions containing a mixture of cells. Captured cells can then be incubated on selective media in order to increase the amount of the target pathogen or, alternatively, they could be used directly for DNA extracion and PCR.

iii) ***Magnetic capture hybridization and PCR (MCH-PCR):*** In MCH-PCR, magnetic beads coated with single stranded DNA probes are used to capture DNA fragments which will be used for PCR. This technique has been successfully used to detect fungi, viruses and bacteria in materials containing PCR inhibitory compounds.

iv) ***Real Time PCR:*** Real time PCR is the most advanced version of PCR wherein the amplification of target sequence can be quantified after each PCR cycle and amplification is pictographically displayed through an attached monitor. The technique can also be implemented in the field by using portable real time PCR machines. Various fluorescence-detection techniques available for RT-PCR are:

1. SYBR green dye based detection
2. TaqMan probes
3. Fluorescent resonance energy transfer (FRET) probes
4. Molecular beacons

***v) Nested PCR:*** In nested PCR two rounds of PCR are performed, the second using a primer set internal to those used in the first round. The nested-PCR was highly useful in detection of *Clavibacter michiganensis subsp. Sepedonicus, Erwinia amylovora, Pseudomonas savastanoi pv. Savastanoi.*

***vi) Multiplex PCR:*** Multiplex PCR is useful for the simultaneous detection of multiple pathogens in a single reaction with more than one set of primers. Multiplex reaction can be run either in a conventional or real time PCR machine. Using multiplex reaction, the total detection time required for individual pathogens (viral, fungal, bacterial) associated with a given crop samples can be reduced.

***vii) Loop-mediated isothermal amplification (LAMP):*** Good approach for amplifying nucleic acid with high specificity, efficiency and rapidity without the need for thermal cycler. LAMP technique has the ability to amplify a target nucleic acid sequence under isothermal conditions with minimal equipment (a water bath or heated block). Simplicity of LAMP-based technique facilitates its use in the field or in less well-resourced settings.

***viii) Repetitive sequence based PCR (Rep-PCR)***: PCR is based on primers corresponding to specific repetitive sequences like enterobacterial repetitive intergenic consensus (ERIC), repetitive extragenic palindromic (Rep) and repetitive BOX elements (BOX) which are dispersed throughout the bacterial genome. ERIC-PCR is an effective method for detection of bacterial pathogens *Xanthomonas* and *Pseudomonas.*

**5. DNA Chip technology (Array technology)**

DNA chips or microarrays represent another DNA-based detection assay that may be applied to test seeds for pathogens. This relatively new technology relies on the unique ability of nucleic acid molecules to hybridize specifically with molecules with complementary sequences. With DNA chip technology, a great number of oligonucleotides are positioned on a small glass or silica surface (chip). Target pathogen DNA is digested into small fragments which are labelled with fluorescent markers and hybridised with oligonucleotides on the DNA chip. The presence of fluorescence indicates if the pathogen of interest is present in the chip specific for that pathogen.

**Microarray detection**

- DNA microarrays are most promising technology for simultaneous detection and identification of many plant viruses
- New emerging method in plant virology
- First described by Gillespie and Spiegelman in 1965

**Principle:** DNA immobilized on a membrane can bind a complementary RNA or DNA strand through specific hybridization

**Types of arrays**

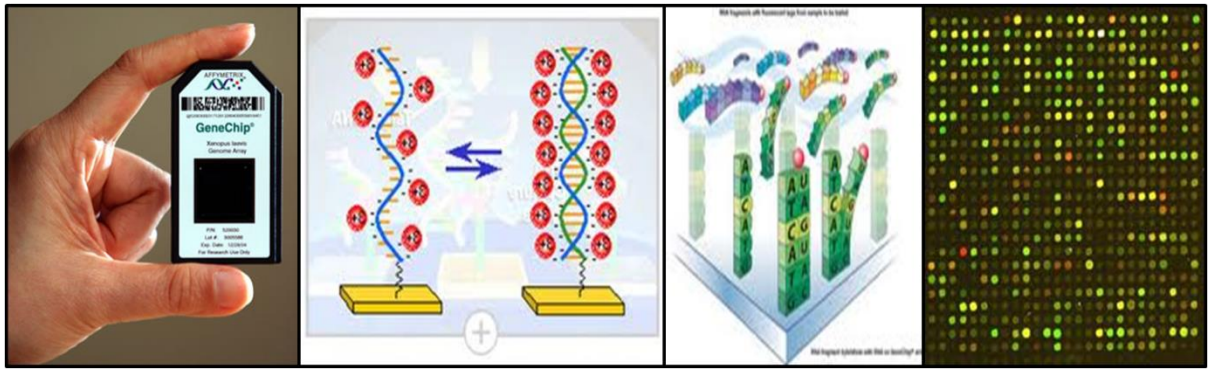
- Macro arrays : contain sample spot size of 300 µm
- Micro Arrays : contain sample spot size of 200 µm

a. Low density: 100 spots/cm2

b. High density: 1000-10000spots/cm2 (DNA chips)

**Steps in microarray technology**

- DNA probes with known nucleotide sequences(CDNA/oligonucleotides)
- Application of DNA to the array
- Target preparation
- Hybridization
- Image detection(scanning/ direct imaging system)
- Analysis of results



# SEED QUALITY ASSURANCE: SPS AND QUARANTINE ISSUES

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## Introduction

Infected or contaminated seed is a primary source of inoculum for a large number of destructive diseases of important food fodder and fiber crops (Neergard, 1977) and is an excellent carrier for the dissemination of phytopathogenic agents to long geographical areas. The devastating effects resulting from diseases and pests introduced along with international movement of planting material, agricultural produce and products are well documented (Khetrapal *et al.*, 2006).

Seeds are the basic input for production. About 90% of the world's food crop is sown using seeds. The major world food crops are barley, beans, maize millet, peanut pulses, rice sorghum, sugarbeet and wheat. These crops are attacked by a large number of pathogens, a majority of which are seed borne. Diseases in general and seed borne diseases in particular are of great economic significance as they are responsible for causing heavy losses. It is a well established fact that seeds are both victim and vehicle of the extensive and complex microflora. There may be hardly any cultivated crop, where atleast one seed transmitted disease is not known. Some of the most important plant pathogens are seed borne and seed transmitted. The various disease causing organisms *i.e.* fungi, bacteria, viruses and even nematodes are carried with the seeds. However, the fungal plant pathogens are the most pre-dominant ones.

In this context Seed Health Tests have become a major component towards sustainable production and productivity. Seed health refers to the presence or absence of disease causing organisms may be fungi, bacteria viruses and animal pests such as eelworms and insects and also abiotic stresses involved to cause deterioration of seed quality. In Seed Health Testing we deal with the detection and identification of seed-borne microorganisms. The objective of seed health testing is to determine the health status of a seed sample so that value of different seed lots can be known. It may elucidate seedling evaluation and causes of poor germination or field establishment and thus supplement germination testing. Thus it is imperative to analyze the seeds for associated pathogen. It has been observed that some saprophytic fungi which produce toxic substances control some important plant diseases, e.g. Species of *Chaetomium* associated with Oat seeds control seedling blight caused by *Fusarium nivale*. By imparting training in this area the beneficiaries can be enriched with various detection and identification methods which in turn will be very useful in keeping seedborne diseases in check. The major purpose of imparting knowledge about seed health testing is to minimise reduction in crop yields by providing useful information regarding presence or absence of

microbes on seeds which may cause loss in germination and vigour of many crops. At the same time it also checks the development of a disease which may become epiphytotic under favourable environmental conditions. Sometimes even saprophytes like *Aspergillus* and *Penicillium* which are not taken as serious concern may be responsible for the loss of viability of seeds during storage. Therefore the seed borne microflora are important in determining seed health.

Plant quarantine is a government endeavour enforced through legislative measures to regulate the introduction of planting materials, plant products, soil, living organisms, etc. in order to prevent inadvertent introduction of pests (including fungi, bacteria, viruses, nematodes, insects and weeds) harmful to the agriculture of a country/ state/ region, and if introduced, prevent their establishment and further spread (Kahn *et al.,* 1989). The plant quarantine measures act as filters against the entry of exotic species and check or delay the introduction of unwanted organisms. Therefore, practicing plant quarantine i.e. protection of plants by observing quarantine measures would go a long way in protecting our agriculture from the ravages of exotic pests and diseases and also in preventing further spread of those already present in a region.

The devastating effects resulting from diseases and pests introduced along with international movement of planting material, agricultural produce and products are well documented. (Khetarpal *et al.,* 2001). The Irish famine of 1845, which forced the people to migrate *en masse* from Europe, was the result of almost total failure of potato crop due to attack of late blight pathogen *(Phytophthora infestans)* introduced from Central America. Coffee rust (*Hemileia vastatrix)* appeared in Sri Lanka in 1875 and reduced the coffee production by > 90% in 1889. The disease entered India in 1876 from Sri Lanka and within a decade, the coffee industry of South India was badly affected. Among the first few countries to promulgate comprehensive plant quarantine regulations were France, Germany, USA, UK and Australia.

In India, heavy losses in grain yield of *Cicer arietinum* crop in states of Haryana, Madhya Pradesh, Punjab and adjoining areas occurred during 1981-82 due to the introduction of virulent biotype of Aschochyta blight from the Middle East. Bunchy top of banana entered India from Sri Lanka and causes loss to banana of over Rs 4 Crores annually (Khetarpal *et al.*, 2004). The dreaded Golden nematode (*Heterodera rostochiensis*) introduced from UK along with exotic seed material in 1960s has been causing severe infestation of potato tubers in the Nilgiris. A noxious weed *Lantana camara* was introduced in 1809 from Central America, which spread to wastelands and pastures causing displacement of native species. All these examples clearly demonstrate that imported seed/ planting material, especially bulk imports, without proper quarantine may result in introduction and establishment of quarantine pests into new areas which may severely damage the crop production and economy of a nation.

**Legislative Measures**

The quarantine measures are of utmost relevance to a country like India whose economy is largely agriculture based. The awareness to quarantine measures in India started in early 20th century when the Indian Government in 1906 ordered compulsory fumigation of imported cotton bales to prevent introduction of Mexican cotton boll weevil (*Anthonomus grandis*). With a view to restrict the entry of exotic pests, pathogens and weeds through regulation of imports, the Government of India legislated the Destructive Insects and Pests (DIP) Act in 1914. This Act has been amended through various notifications issued from time to time also restricted the movement of certain planting material from one state to another state within the country through domestic quarantine. In 1984, a notification was issued under this Act namely Plants, Fruits and Seeds (Regulation of Import into India) Order popularly known as the PFS Order which was revised in 1989 after the announcement of the New Policy on Seed Development by the Government of India in 1988, proposing major modifications for smooth quarantine functioning. This Order has now been superseded by the Plant Quarantine (Regulation for Import into India) Order 2003 which came into force as there was an urgent need to fill-up the gaps in existing PFS order regarding import of germplasm/ GMO's/ transgenic plant material/ bio-control agents etc., to fulfill India's legal obligations under the international Agreements, to protect the interest of the farmers of the country by preventing the entry, establishment and spread of destructive pests, and to safeguard the national bio-diversity from threats of invasions by alien species. Under this Order, the need for incorporation of Additional/ Special declarations for freedom of import commodities from quarantine and invasive alien species (IAS), on the basis of standardized pest risk analysis (PRA), particularly for seed/ planting materials is also taken care of. Further, the scope of plant quarantine activities has been widened with incorporation of additional definitions. The other salient features of the Order are:

- Prohibition on import of commodities with weed/ alien species contamination as per Schedule VIII; & restriction on import of packaging material of plant origin unless treated.
- Provisions included for regulating the import of soil, peat & sphagnum moss; germplasm/ GMOs/ transgenic material for research; live insects/ microbial cultures & biocontrol agents and import of timber & wooden logs.
- Agricultural imports have been classified as (a) Prohibited plant species (Schedule IV); (b) Restricted species where import permitted only by authorized institutions (Schedule V); (c) Restricted species permitted only with additional declarations of freedoms from quarantine/ regulated pests and subject to specified treatment certifications (Schedule VI) and; (d) Plant material imported for consumption/ industrial processing permitted with normal Phytosanitary Certificate (Schedule VII).

- Permit requirement enforced on imports of seeds including flower seeds, propagating material and mushroom spawn cultures.
- Additional declarations being specified in the Order for import of 144 agricultural commodities with specific lists of more than 590 quarantine pests and 61 weed species.
- Notified points of entry increased to 130 from the existing 59.
- Certification fee and inspection charges have been rationalized.

So far, i.e., till November 2007, ten amendments of the Plant Quarantine (PQ) Order 2003 have been notified to the WTO revising definitions, clarifications regarding specific queries raised by quarantine authorities of various countries, with revised lists of crops under the Schedules IV, V VI and VII. The revised list under Schedule VI and VII now include >600 crops/ commodities.

**National Plant Quarantine Set-up**

The Directorate of Plant Protection, Quarantine and Storage (DPPQS) of Ministry of Agriculture is the apex body for implementation of plant quarantine regulation and the PQ order forms the basis of the functioning of the Directorate. It has a national network of 29 plant quarantine stations at different airports (12), seaports (12) and land frontiers (11). In all, three categories of materials are being imported: (a) bulk consignments of grains/ pulses for consumption, (b) bulk consignments of seeds/ planting materials for sowing/ planting, and (c) samples of germplasm in small quantities for research purposes. The PQ Stations under the DPPQS undertake quarantine processing and clearance of consignments of the first two categories. However, National Bureau of Plant Genetic Resources (NBPGR) undertakes the quarantine processing of all plant germplasm and transgenic planting material under exchange. NBPGR has developed well- equipped laboratories and green house complex (Mathur and Lal, 1997). A containment facility of CL-4 level has been recently established for processing transgenics (Khetarpal and Pandey, 2001). Over the years the facilities have been strengthened further and the latest addition is the installation of a transmission electron microscope in 2004.

Out of the three different categories of material being imported, bulk imports for sowing/ planting carry maximum risk as thorough examination and treatment becomes difficult and planting area required is also far too large. Quarantine processing is often restricted to smaller samples derived from them and based on results of these samples the whole consignment is rejected/ detained or released after giving fumigation treatment or fungicidal/ insecticidal treatments. The bulk consignments meant for consumption pose lesser hazards. However, certain small samples meant for research purposes are of immense quarantine importance. These samples usually comprise of germplasm material or wild relatives or landraces of a crop, and are thus more likely to carry diverse biotypes/ races/ strains of the pest/ pathogen. Besides, in case of true seed, generally, risks are more with deep-seated infections than

with surface-borne contamination. However, import of vegetative propagules present a much higher order of risk than true seeds.

A number of pests of great economic and quarantine importance have been intercepted on exotic material, many of which are yet not reported from India viz. insects like *Acanthoscelides obtectus* in *Cajanus cajan*, *Anthonomus grandis* in *Gossypium* spp., *Ephestis elutella* in *Macadamia* nuts and *Vigna* spp., *Quadrastichodella eucalytii* in Eucalyptus, nematodes like *Heterodera schachtii, Ditylenchus dipsaci, D. destructor, Rhadinaphelenchus cocophilus***,** etc. in soil clods and plant debris, fungi like *Claviceps purpurea* in seeds of wheat, barley, etc., *Peronospora manshurica* on soybean, *Fusarium nivale* on wheat, barley and *Aegilops, Uromyces betae* on sugarbeet, bacteria like *Xanthomonas campestris* pv. *campestris* on *Brassica* spp. and v**iruses** like *Barley stripe mosaic virus* in wheat, *Pea seed borne mosaic virus* in pea and fababean, bean yellow mosaic virus in French bean, cowpea mottle virus in cowpea, etc.(Khetarpal *et al.*, 2001)

National Bureau of Plant Genetic Resources (NBPGR) has been empowered by DAC, Govt. of India through Destructive Insects and Pests Act, 1914 (DIP Act, 1914) which has been revised from time to time and presently known as the "Plant Quarantine Order 2003 (Regulation of Import into India)" to undertake the quarantine processing of all germplasm including transgenic planting material under exchange for research purposes, both for public and private sector. The NBPGR, Regional Station at Hyderabad undertakes quarantine inspection and clearance of the PGR meant for research purposes, both for public and private sector of South India.

**Requirements for import of planting material**

***Pre-entry quarantine***:

a) Import Permit is a statutory requirement from the country of import and the conditions/ additional declarations laid on it need to be fulfilled by the country of export.
b) Phytosanitary Certificate is also a statutory requirement and is a proof that the consignment has been examined according to the requirements of the importing country and found to be free from the quarantine pests.
c) Pest risk analysis carried out by the country of import for which desired information is supplied by the country of export.
d) Approval of Post-entry Quarantine (PEQ) Growing Facility is essential as all planting material to be subjected to PEQ inspection as per the PQ Order 2003.

***Post-entry quarantine***

a) Examination of documents to make sure that phytosanitary conditions laid down in the import permit have been taken care of and material is free from exotic pests.

b) Examination of the material after entry, PEQ growing and treatment if required.

***Methodology***

NBPGR imports every year more than 70,000 samples of germplasm and trial material for research use both by public and private sector. The Division of Plant Quarantine at NBPGR has developed procedures for systematic and stepwise processing for detection of pests (Fig. 1): visual and stereo-binocular examination to detect presence of smut and bunt balls, seed galls, ergot sclerotia, rust pustules, spores on the seed; washing test for rusts and downy mildews; blotter method for fungi, bacteria and infectivity, electron microscopy (EM), serological (ELISA) and molecular tests (PCR) to detect seed-borne pathogens including viruses (Chalam and Khetarpal, 2008). Infected materials are either incinerated or salvaged using various salvaging techniques (Agarwal *et al.,* 1998) depending on the category of pathogen(s) detected prior to release.

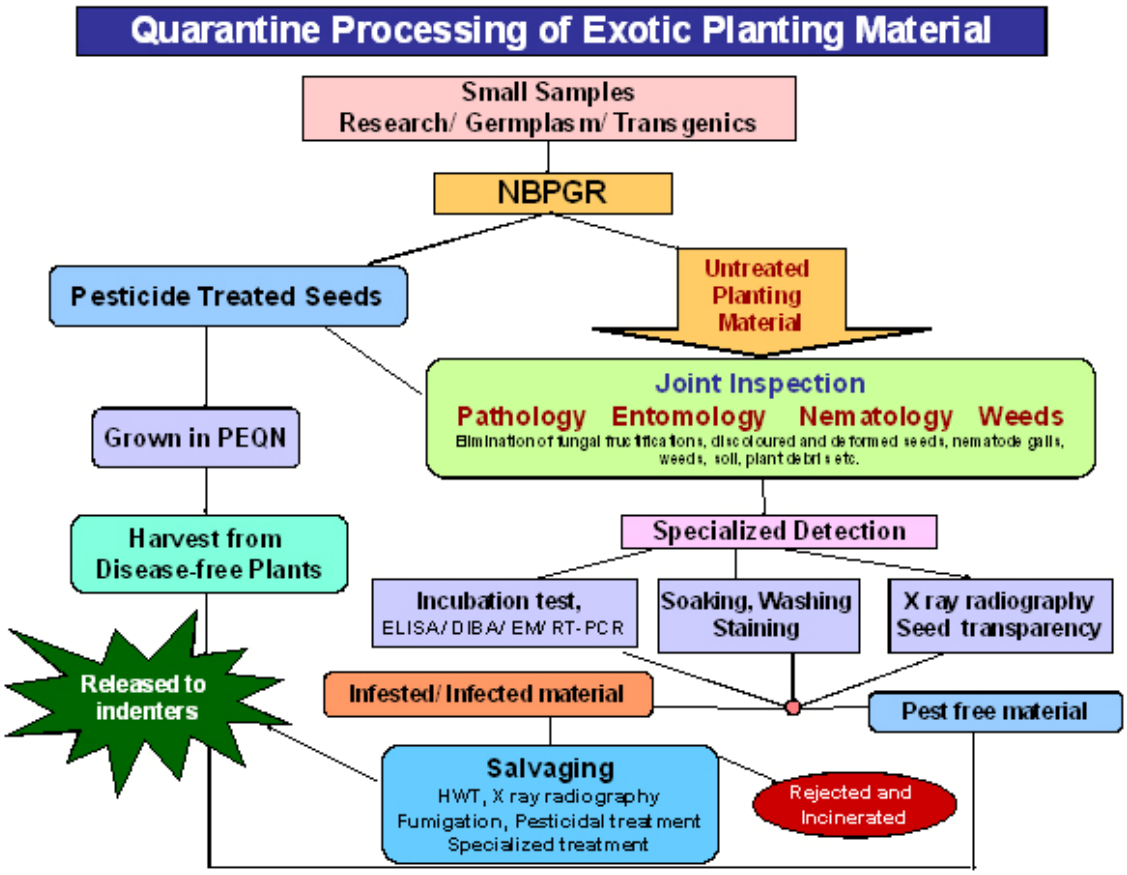


**Fig. 1:** Flowchart of quarantine processing of planting material.

***Results***

Seed-borne pests may result in poor quality seed, loss in germination, development of epiphytotics, distribution of new strains or physiological races of pests along with the seeds and planting material to new geographical areas.

Therefore, critical laboratory examinations with specialized seed health testing methods are conducted and ensured the identification of fungal, bacterial, viral pathogens, insects and mites, nematodes and weed seeds of quarantine importance associated with seeds and other planting materials.

**Present International Scenario**

Exotic germplasm plays an important role in crop breeding and overall agricultural development programmes of India. Diseases and insect pests are among the major causes of low yields and crop failures in our country. Therefore, worldwide movement of germplasm, especially with the liberalisation of food and agriculture trade under the World Trade Organization (WTO) regime, can have a significant impact on the agricultural sustainability.

The external risks associated with imports of germplasm can lead to the possible entry, establishment and spread of exotic seed-borne diseases. Consequently, it can affect the health of plants and inflict damage on an importing country by adversely affecting plant genetic resources, and/or affecting human and animal health through consumption of grains/ plant products.

Although regulations aimed at controlling such diseases can potentially have an impact on the interests of the seed industry, but on the other hand they can help to contain the external risks to plant life and health and to the importing country. This is crucial as the related problems can potentially lead to trade disputes among trading partners.

The recent trade related developments in International activities and the thrust of the WTO Agreements and the Convention on Biological Diversity (CBD) imply that countries need to update their quarantine or plant health services to facilitate pest free import/ export.

***World Trade Organization:***

The establishment of the WTO in 1995 has provided unlimited opportunities for international trade of agricultural products. History has witnessed the devastating effects resulting from diseases and pests introduced along with the international movement of planting materials, agricultural produce and products. It is only recently however, that legal standards have come up in the form of Sanitary and Phytosanitary (SPS) Measures for regulating the international trade. The WTO Agreement on the Application of SPS measures concerns the application of food safety and animal and plant health regulations. It recognizes government's rights to take SPS measures but stipulates that they must be based on science, should be applied only to the extent necessary to protect human, animal or plant life or health and should not arbitrarily or unjustifiably discriminate between members where identical or similar conditions prevail (http://www.wto.org, Khetarpal and Gupta, 2002).

The SPS Agreement aims to overcome health-related impediments of plants and animals to market access by encouraging the "establishment, recognition and application of common SPS measures by different Members". The primary incentive for the use of common international norms is that these provide the necessary health protection based on scientific evidence and improve trade flow at the same time.

SPS measures are defined as any measure applied within the territory of the Member State:

a) to protect animal or plant life or health from risks arising from the entry, establishment or spread of pests, diseases, disease- carrying/ causing organisms;
b) to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease causing organisms in food, beverages or foodstuffs;
c) to protect human life or health from risks arising from diseases carried by animals, plants or their products, or from the entry, establishment /spread of pests; or
d) to prevent or limit other damage from the entry, establishment or spread of pests.

The SPS Agreement explicitly refers to three standard-setting international organizations commonly called as the 'three sisters' whose activities are considered to be particularly relevant to its objectives: the Food and Agriculture Organization (FAO)/ World Health Organization (WHO) Codex Alimentarius Commission, the Office International des Epizooties, and the international and regional organizations operating within the framework of the FAO International Plant Protection Convention (IPPC). They are observers and important contributors to SPS Committee meetings. The IPPC develops the International Standards for Phytosanitary Measures (ISPMs) to provide guidelines on pest prevention, detection and eradication. To date, twenty-four standards have been developed and several others are at different stages of development.

Prior to the establishment of WTO, governments on a voluntary basis could adopt international standards, guidelines, recommendations and other advisory texts. Although these norms remain voluntary, a new status has in effect been conferred upon them by the SPS Agreement. A WTO Member adopting such norms is presumed to be in full compliance with the SPS Agreement.

***Convention on Biological Diversity:***

The Convention on Biological Diversity (CBD) affirms that conservation of biodiversity is a common concern of humankind and reaffirms that nations have sovereign rights over their own biological resources. The treaty states as major objectives the conservation of biological diversity and sustainable use of its components; fair and equitable sharing of the benefits arising out from the use of genetic resources; and appropriate transfer of relevant technologies

(http://www.cbd.org). The quarantine related commitments made by parties to the Convention are:

- to establish means to regulate, manage or control risks associated with use and release of living modified organisms from biotechnology with likely adverse environmental effects;
- to prevent introduction of species from outside a country which could threaten native ecosystems or species;

Although CBD covers all types of organisms (including plants), the scope of IPPC appears broader. The IPPC covers all organisms injurious to plants and plant products, whereas the coverage under the CBD is subject to their sustainable use. The ISPM 11(supplement) on Analysis of Environmental Risks has been developed by IPPC to address the environmental safety issues. This would give an independent framework of rules for WTO members who are not signatories to CBD. In India, the Ministry of Environment and Forests is the nodal agency dealing with IAS for negotiations with CBD, but, it is the Ministry of Agriculture which is the key body dealing with their quarantine, survey and control. Our legislative measures cover diverse aspects of IAS including quarantine, environment protection and trade. However, despite the enforcement of the revised PQ Order 2003 and also the periodically amended Environment (Protection) Act (1986), there is still a need for a cohesive policy and action plan to deal with IAS (Gupta and Khetarpal, 2004).

**Challenges for India under the WTO and CBD Agreements**

- The National Plant Protection Organization needs to be upgraded in terms of work force, infrastructure and capabilities to bring it up to the international standards as the increase in imports and the stipulation of WTO under its SPS Agreement has brought about additional challenges to be faced by quarantine personnel. There is also a dire need to check not only spread of pests but also to promulgate domestic quarantine against certain important pests which have been introduced/ detected in the country in the recent years and which are likely to spread fast.
- In the wake of the SPS Agreement, it has becomes imperative to carry out a PRA for the commodities of trade (Gupta *et al*, 2002). Preparation of a database on endemic pests is a necessity for that matter. Steps taken by Regional Plant Quarantine Station, Chennai in this direction is commendable and needs to be further supported. In addition, a database on the potential quarantine pests for India is being compiled at NBPGR, New Delhi to supplement the information. An effective risk analysis would indeed minimize or eliminate the utilization of techniques for detection of pests and would facilitate the quick release of the material from quarantine based on sound scientific and logical decisions as per WTO norms.
- The export and import of seeds/ planting material are governed by the provisions of Export and Import (EXIM) Policy of Government of India. India

with its strong production infrastructure has considerable potential for exports. With the coming of WTO, India will have better market access of different countries and significant export opportunities for the Indian agriculture sector. Export certification, development of standards for disinfestation and identification of pest free areas (PFAs) are the few fields in which we need to work to boost our exports.

- There is a need to reprioritize our research projects based on critical gaps identified to meet the requirement for export and import. A few that need immediate emphasis are:
  - Survey and surveillance of disease/ pests of national and international importance to have endemic pest data.
  - Generation of a comprehensive epidemiological data on important pests in order to be able to fix *tolerance limits,* to develop PRA and identify PFAs as per the WTO norms.
  - Development of diagnostic protocols using molecular techniques for detection of exotic pests not easily detectable otherwise but are of serious economic concern.
  - Evaluation of the ecological and quarantine risks in import of transgenics and beneficial bio-control agents.
- In the post CBD era, access to germplasm material is not as it was before. Therefore, more efforts need to be made to develop better salvaging techniques so that precious material is available to our researchers.
- The WTO Agreement has endorsed the Cartagena Protocol on Biosafety as under the CBD which has to be followed for the safe movement of transgenic.
- Most of the presently used chemical fumigants viz. methyl bromide would be phased out as per WHO norms in the coming years. Therefore, research on needs to be carried out on use of alternatives like ionizing radiations or physical treatments or other fumigants as quarantine disinfestation treatments.
- There is also a dire need to develop an accessible platform for getting information which is of immense importance for quarantine personnel for their day to day functioning and a valuable source of information for the policy makers, administrators and the industry groups. A web site http://www.plantquarantineindia.com consisting of national database on legislation, quarantine procedures, methodologies, etc. designed by DPPQS needs to be regularly updated.
- The importance of quarantine has increased manifold in the WTO regime and adopting not only the appropriate technique but also the right strategy for pest detection and diagnosis would go a long way in ensuring pest-free exchange of PGR and trade, and is considered the best strategy for managing transboundary movement of pests. It is clear that under the

present international scenario, the plant protection specialists have a major role to play not only in facilitating the import and export in their respective nations but also in protecting the environment from the onslaughts of invasive alien species.

**International standards for phytosanitary measures (ISPMs)**

IPPC has established following standards (1995- 2013)

| S.No | ISPMs | Deals in |
|---|---|---|
| 1 | ISPM 1 | Principles of plant quarantine as related to international trade |
| 2 | ISPM 2 | Guidelines for pest-risk analysis |
| 3 | ISPM 3 | Code of Conduct for the import and release of exotic biological control agents |
| 4 | ISPM 4 | Requirements for the establishment of pest-free areas. |
| 5 | ISPM 5 | A glossary of phytosanitary terms |
| 6 | ISPM 6 | Guidelines for surveillance |
| 7 | ISPM 7 | Export certification system |
| 8 | ISPM 8 | Determination of pest status in an area |
| 9 | ISPM 9 | Guidelines for pest eradication programmes |
| 10 | ISPM 10 | Requirements for the establishment of pest-free places of production and production sites |
| 11 | ISPM 11 | Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms |
| 12 | ISPM 12 | Guidelines for phytosanitary certificates |
| 13 | ISPM 13 | Guidelines for the notification of non- compliance and emergency action |
| 14 | ISPM 14 | The use of integrated measure in a systems approach for pest risk management |
| 15 | ISPM 15 | Guidelines for regulating wood packaging material in international trade |
| 16 | ISPM 16 | Regulated non-quarantine pests: concept and application |
| 17 | ISPM 17 | Pest reporting |
| 18 | ISPM 18 | Guidelines for the use of irradiation as a phytosanitary measure |
| 19 | ISPM 19 | Guidelines on list of regulated pests |
| 20 | ISPM 20 | Guidelines for phytosanitary import regulatory system |
| 21 | ISPM 21 | Pest risk analysis for regulated non-quarantine pests |
| 22 | ISPM 22 | Requirements for the establishment of areas of low pest prevalence |
| 23 | ISPM 23 | Guidelines for inspection |
| 24 | ISPM 24 | Guidelines for the determination and recognition of |

| | | |
|---|---|---|
| | | equivalence of phytosanitary measures |
| 25 | ISPM 25 | Consignments in transit |
| 26 | ISPM 26 | Establishment of pest free areas for fruit flies (Tephritidae) |
| 27 | ISPM 27 | Diagnostic protocols for regulated pests |
| 28 | ISPM 28 | Phytosanitary Treatments for Regulated Pests |
| 29 | ISPM 29 | Recognition of pest free areas and areas of low pest prevalence |
| 30 | ISPM 30 | Establishment of areas of low pest Prevalence for fruit flies (tephritidae) |
| 31 | ISPM 31 | Methodologies for sampling of Consignments |
| 32 | ISPM 32 | Categorization of commodities According to their pest risk |
| 33 | ISPM 33 | Pest free potato (solanum spp.) Micropropagative material and minitubers For international trade |
| 34 | ISPM 34 | Design and operation of post-entry Quarantine stations for plants |
| 35 | ISPM 35 | Systems approach for pest risk management of fruit flies (Tephritidae). |
| 36 | ISPM 36 | Outlines criteria for identification/application of integrated measures for production and trade of plants for planting. |
| 37 | ISPM 37 | Determination of host status of fruit to fruit flies (Tephritidae) |
| 38 | ISPM 38 | International Movemnt of seeds added in May 2017 |

Draft ISPM 39: International movement of wood prepared but not approved.

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# IMPORTANCE AND USE OF TOLERANCES IN SEED TESTING

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

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

For the above situations and to ensure that analysis conducted within the laboratory is accurate, it in necessary at the part of the analyst to make sure that the result being reported is valid/reproducible. The application of an appropriate statistical method to test the results of seed testing enables the analyst to determine the validity of results within a calculated range of limits, the amount of this range in called the 'Tolerance'. For seed quality determination, replicated test is conducted from same sample or different samples drawn from the same lot and replicated tests are conducted in one laboratory or different laboratories. It is generally observed that the test results may not be the same. The differences among the result are compared with non-significant permissible value supported with the statistical evidence or calculated range of limits. In seed testing, this maximum non-significant calculated range of limit or expected variation is called "Tolerance Number" and a series of such tolerance numbers is called 'Tolerance Table'. Tolerance tables

are used for testing the significance of the precise estimates for (a) to describe the seed quality (b) to decide if the estimate agrees well enough with another estimate or specification. In other words the object of using tolerances is to provide a means of assessing whether or not the variation within the test results or between the tests is sufficiently wide to raise doubt about the accuracy of results.

**A. Sources of Variation in Test Results**:

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

**B. Basic Assumptions for Using Tolerances:**

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

**C. Where to Apply Tolerances:**

**C.1. Within Seed Testing Laboratory:**

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

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

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

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

**C.2. For Certification:**

**(a)** The Central Seed Committee (CSC), Government of India has prescribed the minimum seed certification standards for various crops. As a practice these standards are also taken as such for label information, even if the seed analysis

results may be higher than the prescribed standards, the actual prescribed standards are only mentioned on the table.

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

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

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

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

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

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

### C.3. Outside seed testing laboratory:

**(a) Seed law enforcement- Label prescription:**

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

**(b) Seed law enforcement -Seed standard prescription:**

The central seed committee has prescribed crop wise minimum seed certification standards class wise for labeling seeds. At present the seed

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

**D. How to Use Tolerance Table:**

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

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

**(a) How to Use For Purity Analysis Results:**

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

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

| **Average analysis of two half samples or two whole sample** | | **Tolerance for differences between** | |
|---|---|---|---|
| | | **Half working samples** | **Whole working samples** |
| 1 | 2 | 3 | 4 |
| 99.95-100.00 | 000-0.04 | 0.23 | 0.16 |
| 99 90-99 94 | 005-009 | 0.34 | 0.24 |
| 99.85-99.89 | 0.10-0.14 | 0.42 | 0.30 |
| 99.80-99.84 | 0.15-0.19 | 0.49 | 0.35 |
| 99.75-99.79 | 0.20-0.24 | 0.55 | 0.39 |
| 99.70-99.74 | 0.25-0.29 | 0.59 | 0.42 |
| 99.65-99.69 | 0.30-0.34 | 0.65 | 0.46 |
| 99.60-99.64 | 0.35-0.39 | 0.69 | 0.49 |
| 99.55-99.59 | 0.49-0.44 | 0.74 | 0.52 |
| 99.50-99-54 | 0.45-0.49 | 0.76 | 0.54 |
| 99.40-99.49 | 0.50-0.59 | 0.82 | 0.58 |
| 99.30-99.39 | 0.60-0.69 | 0.89 | 0.63 |
| 99.20-98.29 | 0.70-0.79 | 0.95 | 0.67 |
| 99.10-99.19 | 0.80-0.89 | 1.00 | 0.71 |
| 99.00-99.09 | 0.90-0.99 | 1.06 | 0.75 |
| 98.75-98.99 | 0.00-1.24 | 1.15 | 0.81 |

| | | | |
|---|---|---|---|
| 98.50-98.74 | 1.25-1.49 | 1.26 | 0.89 |
| 98.25-98.49 | 1.50-1.74 | 1.37 | 0.97 |
| 98.00-98.24 | 1.75-1.99 | 1.47 | 1.04 |
| 97.75-97.99 | 2.00-2.24 | 1.54 | 1.09 |
| 97.50-97.74 | 2.25-2.49 | 1.63 | 1.15 |
| 97.25-97.49 | 2.50-2.74 | 1.70 | 1.20 |
| ….* | …. | …. | …. |
| 82.00-83.99 | 16.00-17.99 | 3.90 | 2.76 |
| 80.00-81.99 | 18.00-19.99 | 4.07 | 2.88 |
| 78.00-79.99 | 20.00-21.99 | 4.23 | 2.99 |
| 76.00-77.99 | 22.00-23.99 | 4.37 | 3.09 |
| 74.00-75.99 | 24.00-25.99 | 4.50 | 3.18 |
| 72.00-73.99 | 26.00-27.99 | 4.61 | 3.26 |
| 70.00-71.99 | 28.00-29.99 | 4.71 | 3.33 |
| 65.00-69.99 | 30.00-34.99 | 4.86 | 3.44 |
| 60.00-64.99 | 35.00-39.99 | 5.02 | 3.55 |
| 50.00-59.99 | 40.00-49.99 | 5.16 | 3.65 |

*. …Indicates the other values in the series

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

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

*. …Indicates the other values in the series

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

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

*. …Indicates the other values in the series

**Examples of Use of Tolerance Table for Purity Analysis Results:**

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

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

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

**(b) How to Use For Germination Test Results:**

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

distinguishing between normal and abnormal seedlings (v) fungi or bacteria (vi) chemicals on the seed (viii) inaccurate counting (viii) non random selection of seeds for test or (ix) actual change in the per cent germination between tests.

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

| **Average percent germination** | | **No. replicates of 100 seeds** | | |
|---|---|---|---|---|
| | | **4 rep.** | **3 rep.** | **2 rep.** |
| **1** | **2** | **3** | **4** | **5** |
| 99 | 2 | 5 | 4 | - |
| … | … | … | … | … |
| 97 | 4 | 7 | 6 | 5 |
| … | … | … | … | … |
| 93-95 | 7-8 | 10 | 9 | 8 |
| … | … | … | … | … |
| 78-80 | 21-23 | 16 | 15 | 13 |
| 77 | 24 | 17 | 15 | 13 |
| 73-76 | 25-28 | 17 | 16 | 14 |
| … | … | … | … | … |
| 64-66 | 35-37 | 19 | 18 | 15 |
| … | … | … | … | … |
| 51-55 | 46-50 | 20 | 18 | 14 |

*. …Indicates the other values in the series

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

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

*. …Indicates the other values in the series

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

| **Average Percent** | **Tolerance** |
|---|---|

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

*. …Indicates the other values in the series

**Examples of Use of Tolerance Table for Germination Test Results:**

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

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

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

**C. Use of Tolerances under Seed Law Enforcement Programme:**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**Comparing the laboratory results with the label information:-**

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

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

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

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

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

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

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

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

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

**Comparing the laboratory result with the minimum standards prescribed :**

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

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

Laboratory result: 97.0 Standard : 98.0 Difference : 1.0

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

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

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

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

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

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

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

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

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

**A=** Average of laboratory's result and label information. **B=** Tolerance Level
Hand book of Tolerances and Measures of Precision for seed Testing, Table F2, only 5% Probability column is used.

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

| **Average percent Germination** | | **4Tests** | **3Tests** | **2Tests** |
|---|---|---|---|---|
| **A** | **B** | **C** | **D** | **E** |
| 99 | 2 | 2 | 2 | 1 |
| 98 | 3 | 3 | 2 | 2 |
| 97 | 4 | 3 | 3 | 2 |
| 96 | 5 | 3 | 3 | 3 |
| 95 | 6 | 4 | 3 | 3 |
| 94 | 7 | 4 | 4 | 3 |
| 93 | 8 | 4 | 4 | 4 |
| 92 | 9 | 5 | 5 | 4 |
| 91 | 10 | 5 | 4 | 3 |
| 90 | 11 | 5 | 5 | 4 |
| 89 | 12 | 5 | 5 | 4 |
| 88 | 13 | 6 | 5 | 4 |
| 87 | 14 | 6 | 5 | 4 |
| 86 | 15 | 6 | 6 | 5 |
| .. | .. | .. | .. | .. |
| | | | | |
| 78 | 23 | 7 | 7 | 6 |
| .. | .. | .. | .. | .. |
| 75 | 26 | 8 | 7 | 6 |
| 67 | 34 | 8 | 8 | 6 |
| .. | .. | .. | .. | .. |
| 63 | 38 | 8 | 8 | 6 |
| 62 | 39 | 9 | 8 | 6 |
| 60 | 41 | 9 | 8 | 6 |
| 59 | 42 | 9 | 8 | 7 |
| .. | .. | .. | .. | .. |
| 51 | 50 | 9 | 8 | 7 |

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

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

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

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

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

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

**A= Standard** **B= Rejected**

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

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

| **Average percent Germination** | | **4Tests** | **3Tests** | **2Tests** |
|---|---|---|---|---|
| **A** | **B** | **C** | **D** | **E** |
| 99 | 2 | 1 | 1 | 2 |
| 98 | 3 | 2 | 1 | 3 |
| 97 | 4 | 2 | 1 | 3 |
| 96 | 5 | 2 | 2 | 4 |
| 95 | 6 | 3 | 2 | 4 |
| 94 | 7 | 3 | 2 | 4 |
| 93 | 8 | 3 | 2 | 5 |
| 92 | 9 | 3 | 2 | 5 |
| 91 | 10 | 4 | 3 | 6 |
| .. | .. | .. | .. | .. |
| 86 | 15 | 5 | 3 | 7 |
| .. | .. | .. | .. | .. |
| 82 | 19 | 5 | 4 | 7 |
| 81 | 20 | 5 | 4 | 8 |
| .. | .. | .. | .. | .. |
| 79 | 22 | 6 | 4 | 8 |
| .. | .. | .. | .. | .. |
| 75 | 26 | 6 | 4 | 9 |
| .. | .. | .. | .. | .. |
| 70 | 31 | 7 | 5 | 9 |
| 69 | 32 | 7 | 5 | 10 |
| .. | .. | .. | .. | .. |
| 59 | 42 | 7 | 5 | 11 |
| .. | .. | .. | .. | .. |
| 57 | 44 | 8 | 5 | 11 |
| .. | .. | .. | .. | .. |
| 51 | 50 | 8 | 5 | 11 |

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

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# ISTA ACCREDITATION-PROCEDURE AND BENEFITS

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Though the importance of quality seed was realized with the inception of agriculture; however, the concept of seed quality on scientific lines, gained importance during 1989, when Professor Friedrich Nobbe in Germany advocated that the seeds must be tested before sowing. Nobbe's hypothesis was based on scientific investigations made by him on the vegetable and flower seed samples offered for marketing in European Countries. This gave birth to the establishment of seed testing laboratories in European countries, USA and Canada during late eighties and early nineties. The establishment and development of seed testing laboratories in the developed world generated tremendous impact on the seed trade and the development of seed testing procedures. The International Seed Testing Association (ISTA) was established in 1924 during the 4th International Seed Testing Congress held in Cambridge, United Kingdom with the primary aim to develop, adopt and publish standard procedures for sampling and testing seeds, and to promote uniform application of these procedures for evaluation of seeds moving in international trade. ISTA is engaged in the development of standardized seed testing procedures, which are available in the form of International Rules for Seed Testing and its members work together to achieve their vision of 'Uniformity in seed quality evaluation worldwide' and produces internationally agreed rules for seed sampling and testing, accredits laboratories, promotes research, provides international seed analysis certificates and training, and disseminates knowledge in seed science and technology. This facilitates seed trading nationally and internationally, and also contributes to food security. The first set of International Rules of Seed Testing were framed and published by ISTA during 1931. The ISTA rules are henceforth being updated annually; which contain seed testing protocols of large number of plant species and forms the basic reference book for all kinds of seed testing activities and also for the international seed trade.

ISTA is an independent body and acts free from economic interest and political influence; it is unbiased, objective and fair. Furthermore, the hitherto unsurpassed expertise of ISTA is based on the non-profit cooperation of the international community of approximately 400 experienced, competent and energetic seed scientists and analysts. ISTA membership consists of member laboratories and sampling entities, personal members, associate members and industry members throughout the world. The membership is a collaboration of seed scientists and seed analysts from universities, research centres and governmental, private and company seed testing laboratories around the world. ISTA values and promotes the diversity of membership, which forms the basis

for its independence from economic and political influence. As of January 1st 2018, the membership of ISTA counts 225 Member Laboratories, 35 Personal Members and 63 Associate Members in 83 countries/distinct economies. Of these, 137 Member Laboratories are accredited by ISTA and entitled to issue ISTA International Seed Analysis Certificates. ISTA has stood for uniformity in seed testing for more than 80 years, which is managed and directed by an Executive Committee. According to Article 15 (a) of the Articles of ISTA, the Executive Committee shall consist of the President, Vice-President and Immediate Past President, together with eight members-at-large. All Executive Committee members are Designated Members of ISTA. The finances and administration of the association is managed by the ISTA Secretariat, based in Switzerland and lead by the Secretary General with 8 staff members. There are 17 subject-focused Technical Committees, which are constituted from amongst approximately 400 members and many of which are active in more than one committee. These Technical Committees are responsible for the development of new methodologies for seed testing and each committee is headed by a chair and vice chair. ISTA Technical Committees include Advanced Technologies, Bulking and Sampling, Editorial Board of Seed Science and Technology, Flower Seed Testing, Forest Tree and Shrub Seed, Germination, GMO, Moisture, Nomenclature, Proficiency Test, Purity, Rules, Seed Health, Seed Science Advisory Group, Statistics, Seed Storage, Tetrazolium, Variety, Vigour. The methods of the ISTA Rules are being validated, internationally harmonized and voted on by the ISTA membership.

**The objectives of the Association are:**

**(a)** The primary purpose of the Association is to develop, adopt and publish standard procedures for sampling and testing seeds, and to promote uniform application of these procedures for evaluation of seeds moving in international trade.

**(b)** The secondary purposes of the Association are to promote research actively in all areas of seed science and technology, including sampling, testing, storing, processing, and distributing seeds, to encourage variety (cultivar) certification, to participate in conferences and training courses aimed at furthering these objectives, and to establish and maintain liaison with other organisations having common or related interests in seed.

The ISTA membership consists of Member Laboratories, Personal Members, Associate and Industry Members.

**Member Laboratory**: A Member Laboratory is a laboratory engaged in the testing of seed which supports the Association and its objectives and is admitted by the Association. The Member Laboratory can participate in the ISTA Proficiency Test Programme, and once accredited by ISTA, be authorised to issue ISTA International Seed Analysis Certificates. Once admitted as a

member, the Member Laboratory is entitled to free online multi-user access to the 'International Rules for Seed Testing', and a free online multi-user subscription to 'Seed Science and Technology'. The Member Laboratory also receives, through its Personal Member, ISTA Handbooks and proceedings published after admission, and the ISTA newsletter 'Seed Testing International'. Additional copies of ISTA publications which pre-date the membership can also be purchased at the Member's reduced price. A Member Laboratory membership also includes that for one Personal Member, whose postal address has to be the same as that of the laboratory. A Member Laboratory usually nominates the Personal Member to be its representative in the affairs of the Association. The Personal Member may be designated by their Designated Authority as a Designated Member, and be authorised to execute the country/distinct economy's voting right on behalf of its Government at ISTA Ordinary Meetings. All staff members of the Member Laboratory receive priority for any ISTA event and can benefit from the reduced membership registration fees for ISTA Congresses, Ordinary Meetings, Symposia and Workshops.

**Personal Member**: A Personal Member is a person engaged in the science and practice of seed testing or in the technical control of such activities and who supports the Association and its objectives and is admitted by the Association. A Personal Member is entitled to free online single-user access to the 'International Rules for Seed Testing', and a free online single-user subscription to 'Seed Science and Technology'. A Personal Member also receives ISTA Handbooks and proceedings that are published after admission as Member, and the ISTA newsletter 'Seed Testing International'. Additional copies of ISTA publications which predate the membership can also be purchased at the Member's reduced price. A Personal Member receives priority at any ISTA event and can benefit from the reduced membership registration fees for ISTA Congresses, Ordinary Meetings, Symposia and Workshops. A Personal Member may be designated by their Designated Authority as a Designated Member, and be authorised to execute the country/distinct economy's voting right on behalf of its Government at ISTA Ordinary Meetings.

**Associate Member:** An Associate Member is a person who is not a Personal Member, but who supports the Association and its objectives, and is admitted by the Association. An Associate Member receives 'Seed Testing International' and has priority for membership of an ISTA Technical Committee over individuals who are not ISTA Members. An Associate Member can purchase the ISTA Handbooks, proceedings, and single-user online access to the 'International Rules for Seed Testing' at the reduced membership price, and can benefit from the reduced membership registration fees for ISTA Congresses, Ordinary Meetings, Symposia and Workshops. An Associate Member cannot

become a Designated Member and therefore cannot vote at ISTA Meetings or hold office in the Association.

**Industry Member**: An Industry Member is an organisation which supports the Association and its objectives, and through paying an annual fee, provides sponsorship to the Association. An Industry Member is any entity which supports the Association and its objectives, pays an appropriate annual fee to the Association, and is admitted by the Association. The Industry Membership fee depends on the number of employees.

**The benefits accrued from obtaining ISTA membership are:**

- Provides the basis for ensuring the trade of quality seed by developing standard seed testing methods
- Provides a platform for research and cooperation between seed scientists worldwide
- Promotes research and provides the opportunity for publishing and distributing of the technological data
- Guarantees worldwide harmonised, uniform seed testing through the Accreditation, Proficiency Test and Auditing Programmes
- Provides services and professional development programmes for furthering the education and experience of seed analysts around the world
- Provides an unbiased voice in the seed industry

As an authority in seed science and technology, ISTA continues its role as the developer of seed testing methods. Its major achievements and services provided till date include:

- ISTA International Rules for Seed Testing, guaranteeing worldwide annually updated, harmonized and uniform seed testing methods.
- ISTA Accreditation Programme, including Accreditation Standard, Proficiency Testing Programme and Auditing Programme guaranteeing worldwide harmonized and uniform seed testing.
- Issuance of the ISTA International Seed Lot Certificates by officially independent ISTA accredited and authorised laboratories.
- Promotion of research, training, publishing and information in all areas of seed science and technology and cooperation with related organisations such as ISF, OECD, UPOV and many others.

**ISTA Accreditation:**

Accreditation is defined as the procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific task. It is a process through which a laboratory's technical competence is verified through assessment by an experienced audit team against established audit criteria The aim of ISTA Accreditation is to verify if a seed testing laboratory is

technically competent to carry out seed testing procedures in accordance with the 'ISTA International Rules for Seed Testing'. Accredited laboratories must show that they run a quality management system fulfilling the requirements of the ISTA Accreditation Standard. The laboratories accredited by ISTA are authorized to issue ISTA Seed Lot and Sample Certificates**.** By reporting seed test results on ISTA Seed Lot Certificates, the issuing laboratory assures that the sampling and testing has been carried out in accordance with the ISTA Rules. . The ISTA Certificates provide an assurance that the test results are reproducible, true and represent the quality of the seed. An accredited laboratory must satisfy two additional conditions before it can be authorized to issue ISTA certificates:

1. The laboratory must not have any financial interest in the production, processing and/or distribution of seeds.
2. The laboratory must have taken part, usually for at least three years, in the ISTA referee testing programme with conclusive results.

The certificate shall be issued only on forms obtained from the International Seed Testing Association.

The International Seed Analysis Certificates are available only for accredited member laboratories of ISTA. They must only be issued in accordance with the ISTA Rules currently in force. ISTA Certificates are accepted by many authorities and are mentioned into the Seed Act of several countries. These certificates are of two kinds:

**A) Orange International Seed Lot Certificate:** This is issued when both sampling from the lot and testing of the sample are carried out under the responsibility of an ISTA-accredited laboratory. The sample is drawn officially from the lot under the authority of a member station. The lot is sealed, labelled and tested for seed quality attributes by the same member station. The orange certificate shall have the following information:

1. Name of the issuing station
2. Name of the sampling and sealing agency
3. Official mark and seal of the lot
4. Number of containers in the lot
5. Date of sampling
6. Date of sample received by testing station
7. Date of issuance of certificate
8. Testing station's test number
9. Result of tests
10. The following statement signed by the appropriate authority of the issuing station "I certify that sampling, sealing and testing have been carried out in accordance with the International Rules for Seed Testing of ISTA and that the tests have been made at the official Station authorised by the ISTA to issue international Seed Analysis Certificate".

**B) Blue International Seed Sample Certificate:** This is issued when the sampling from the lot is not under the responsibility of an ISTA-accredited laboratory. It refers to the sample submitted for testing and shall be printed on blue paper. Therefore, the results obtained by the ISTA-accredited laboratory apply only to the sample and not to the seed lot the sample was taken from. The information on blue certificate would be the same as given above except that of items (2) and (3) would be deleted and the statement given above in (10) shall be as follows: " I certify that testing has been carried out accordance with the International Rules for seed Testing of the 1STA and that the tests have been made at an official station authorized by the 1STA to issue International Certificates".

These certificates can be purchased from the ISTA Secretariat, contacting audit(at)ista.ch address, with a minimum quantity to be ordered is one box containing 100 certificates sheets. The price per sheet is in Swiss Francs CHF 3.25 and it is the same for Orange and for Blue certificates. About 200000 TA Orange International Seed Lot Certificates and Blue International Seed Sample Certificates are issued every year, facilitating seed trading internationally.

**The definitions and abbreviations with respect to ISTA Accreditation are given below:**

**Accreditation:** Procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks.

**Accreditation body:** Body that conducts and administers an accreditation system and grants accreditation.

**Audit:** Systematic, independent and documented process for obtaining audit evidence and evaluating it objectively to determine the extent to which audit criteria are fulfilled.

**Auditor/Assessor:** Person with competence to conduct an audit.

**Authorisation:** Approval by the ISTA Executive Committee that an ISTA accredited laboratory may issue ISTA International Seed Analysis Certificates.

**ISTA Laboratory Accreditation Standard:** Document provided by the ISTA Secretariat and approved by the Executive Committee where requirements of the quality management systems are laid down. Seed testing laboratories are assessed against this standard.

**ISTA Rules:** ISTA International Rules for Seed Testing, published by the Association

**Laboratory Proficiency Test Programme:** Determination of laboratory testing performance by means of inter-laboratory comparisons.

**On-site assessment:** Part of the audit conducted by an ISTA audit team to verify compliance of the current quality management system with the requirements of the ISTA Laboratory Accreditation Standard which takes place in the premises of the laboratory.

**Quality Manual:** Document specifying the quality management system of an organisation.

**Re-accreditation audit:** Audit conducted every three years after the first audit to verify maintenance of the quality management system.

**Repeat audit:** Additional assessment conducted after a (re-)accreditation audit to verify the suitability of corrective actions taken to address audit findings. This might be necessary when major non-compliances occur and removal cannot be verified through submission of documents.

**Scope of accreditation:** The scope of accreditation gives details of activities for which the laboratory is accredited in terms of methods in the current version of the ISTA Rules and species mentioned there, including methods for which a laboratory can be accredited under the Performance Based Approach. It cannot comprise methods described only in ISTA Handbooks or Working Sheets. The scope of accreditation must be documented and communicated to the staff members.

**The responsibilities/ obligations of an Accredited Laboratory**

- To advise the ISTA Secretariat in advance of any significant changes to its ownership, affiliation, organisation, location, or any other matter relevant to its status as an ISTA accredited member laboratory. The ISTA Secretariat will then assess the effect of such changes, on a case-by-case basis, and if accreditation may be maintained or whether maintenance is dependent on the result of an audit.
- To provide any additional documentation and/or survey information relating to its accreditation, as requested by the ISTA Secretariat.
- To continuously abide by the ISTA Laboratory Accreditation Standard once accreditation is granted.
- To discontinue immediately the use of ISTA Certificates and return any unused ISTA Certificates and the Certificate of Accreditation to the ISTA Secretariat in the event of withdrawal or termination of accreditation. The conditions for termination, suspension and withdrawal of accreditation are laid down in 'Procedures for Termination, Suspension and Withdrawal of ISTA Accreditation' obtainable from the ISTA Secretariat.

**Register of Accredited Laboratories:** A directory of accredited laboratories is published by ISTA, including the names of the laboratories and their scope of accreditation. Details of every new or re-accredited laboratory are published in Seed Testing International.

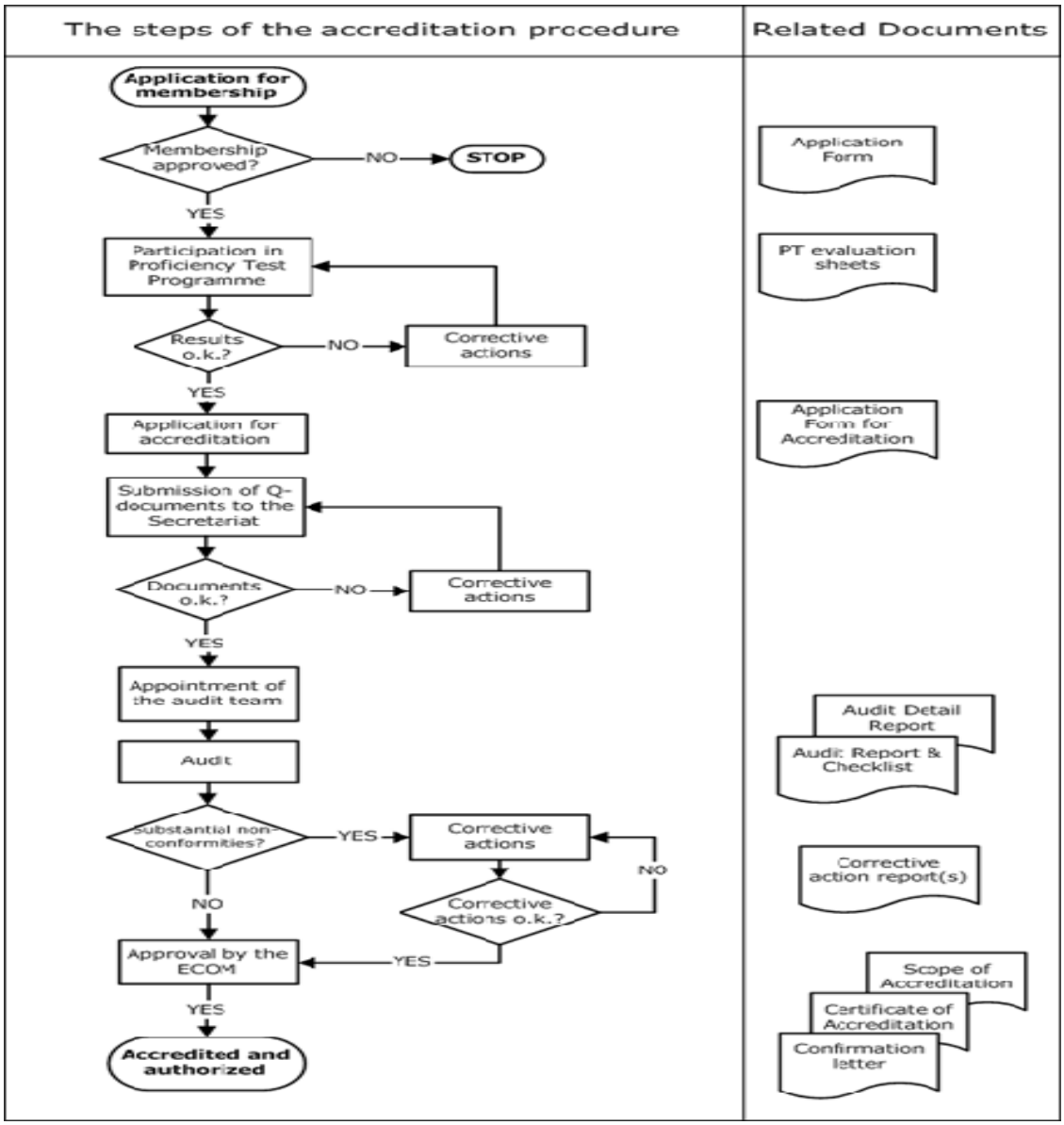
**Keeping of Records**: The documents concerning the accreditation process for individual laboratories are kept by the ISTA Accreditation Department.

**Confidentiality**: All information and documents regarding current accreditations and their results are kept confidential.

**Re-assessment of Accredited Laboratories:** Accreditation is valid for three years, starting from the date of the audit. A re-assessment should take place within three months of the third anniversary of accreditation.

**Use of ISTA Logo and the Way of Referring to the Accreditation Granted**: ISTA accredited laboratories may refer to their accreditation status on letters and reports. The use of the ISTA logo, which is a registered trade mark, by members for example for public relations' purposes is restricted following the regulations of the Association.

**The steps in the Accreditation process are given below:**



**Relevant Accreditation Documents for the Accreditation of laboratories for the testing of specified traits under a performance based approach**:
During the Ordinary Meeting 2013 in Antalya, Turkey, the proposed ISTA rules for testing of GMO traits (Chapter 19) was adopted. This has come into effect on January 1, 2014.By following these rules laboratories can become accredited for the testing of GM traits in seed under the performance-based approach.

**Scope of the ISTA Proficiency Test (PT) Programme**: All ISTA member laboratories are eligible to participate in all PT rounds of the ISTA PT Programme, an Inter-laboratory ring test to access their technical competence. The samples are tested according to standard methods and statistically compared (Z-Score). This provides for the evaluation of lab testing performance, detection of systematic errors in testing and determination of trends in testing performance. It is mandatory for ISTA accredited member laboratories (depending on their scope of accreditation) and voluntary for non-accredited laboratories who want to benchmark themselves with accredited laboratories and prepare themselves for accreditation in the future. The goal is not to identify the best seed testing laboratory in the world, but to identify those laboratories that do not meet the minimum standard of performance that is reasonably expected from an ISTA accredited laboratory and to determine if such laboratories are taking reasonable corrective action to bring their performance standard to at least the minimal level. Non-members of ISTA may participate in the ISTA PT Programme for a flat fee determined and published by the Association. Participation comprises shipment of samples, statistical analysis and reporting to the participating laboratory. Non-members who wish to participate are required to contact the ISTA Secretariat.

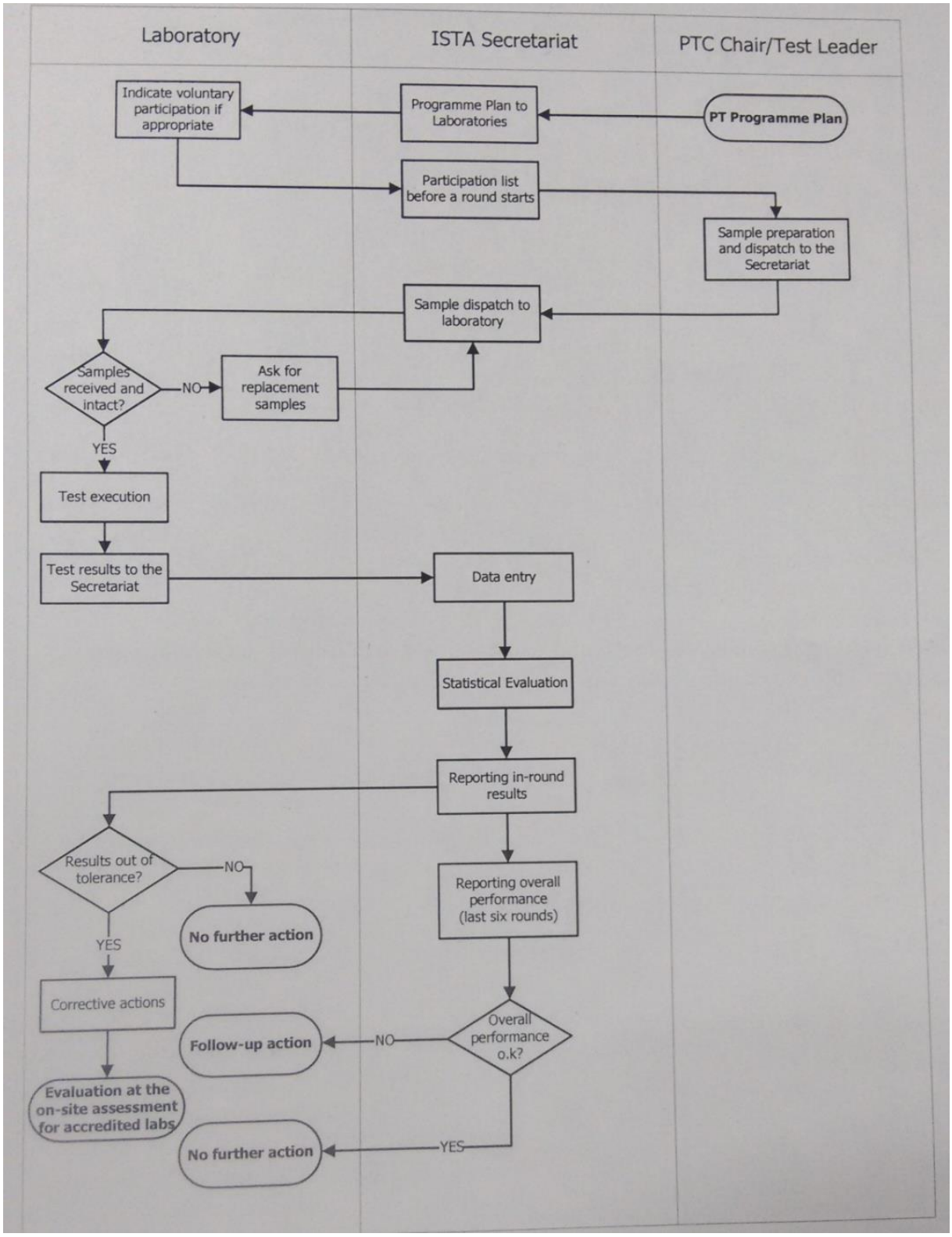
**Schedule**: Regular PT rounds are performed three times per year. Normally, a test round starts 1st of April, August and December each year with the shipment of the samples as indicated in the Programme Plan. There are six designated crop groups; a representative species_of the each crop group is tested. Each round is made up of three samples/lots for each test (or group of tests) that are analysed for e.g. purity, other seed determination (OSD), germination, moisture, and/or Tetrazolium as applicable. Occasionally, Vigour and Seed Health may be included. PTs for tests other than those mentioned are currently organised by the GMO and Seed Health Committee. The updated information about the upcoming GMO Proficiency Test and Seed Health Proficiency Test rounds is available on their committee websites. ISTA members are automatically informed about upcoming GMO and Seed Health Proficiency Test rounds by email.

**Programme Plan**: The ISTA Proficiency Test Committee (PTC), comprising the Chairman and the PT Leaders, select the particular species to be used for the

programme over a three year time span between two ISTA Congresses. The species to be used are chosen to represent a size and germination type group and are intended to be generic in representing a crop group. They are species listed in the ISTA Rules Table 2A Part I and III. All species of Table 2A may be part of a laboratory's scope of accreditation. If a laboratory is accredited for one or several of the species mentioned in a crop group, participation in a test round which tests another species of the same crop group is mandatory. A representative species for each Crop Group should be chosen at least once within six rounds. At the beginning of each PT period, generally after an ISTA Congress, the laboratory will receive the Programme Plan. New member laboratories will receive the Programme Plan together with the membership confirmation letter. Non-accredited laboratories that wish to participate as volunteers will be requested to indicate in which test rounds they wish to participate by returning the completed form to the ISTA Secretariat. Accredited laboratories whose scope does not oblige them to participate in every test round may also indicate the rounds in which they wish to participate as volunteers. Results of voluntary test rounds will be excluded from the accredited laboratory's overall rating. Test rounds are identified by a unique numbering system that is as follows: last two numbers of the year in which the test is performed, a number indicating the round of that year plus an abbreviation of the species tested. Hence, 07-2 *M.sat* means that it is the second round of year 2007 and the species tested is *Medicago sativa*. This number is applied and referred to in communication to quickly identify the test round concerned.

**Participants:** Before a round starts, the list of participants is generated on the basis of the data available in the Secretariat's data base. This list is made up of laboratories obliged to participate due to their specific scope of accreditation and laboratories having indicated their interest. The mandatory status of every laboratory is determined on the basis of the approved scope of accreditation. The scope of accreditation is defined as described in the document Acc-D-07-Scope of Accreditation Policy.

Accredited laboratories are reassessed in regular intervals to examine if they continuously abide by the ISTA accreditation requirements. The accredited laboratories are obliged to participate in relevant proficiency test rounds according to the testing capabilities. The failure in the proficiency test programme may lead to suspension of the accreditation.


Laboratory
ISTA Secretariat
PTC Chair/Test Leader
Indicate voluntary participation if appropriate
Programme Plan to Laboratories
PT Programme Plan
Participation list before a round starts
Sample preparation and dispatch to the Secretariat
Sample dispatch to laboratory
Samples received and intact?
NO
Ask for replacement samples
YES
Test execution
Test results to the Secretariat
Data entry
Statistical Evaluation
Reporting in-round results
Results out of tolerance?
NO
No further action
YES
Corrective actions
Reporting overall performance (last six rounds)
Overall performance o.k?
NO
Follow-up action
Evaluation at the on-site assessment for accredited labs
No further action
YES

**Crop groups for purity, Other seed determination and germination tests Group 1 to 6 covers species/genera of Table 2a, Part 1.**

| Crop group | Size | Species belonging to the following genera |
|---|---|---|
| 1 (grasses) | A | *Poa pratensis, Poa trivialis, Dactylis* |
| | B | *Agrostis, Anthoxanthum, Crambe, Cynodon, Cynosurus, Deschampsia, Eragrostis, Holcus, Phleum, Poa, Schizachyrium* |
| | C | *Agropyron, Alopecurus, Arrhenatherum, Beckmannia, Bromus, Ehrharta, Elymus, Elytrigia, Festuca, XFestulolium, Koeleria, Lolium, Pascopyrum, Phalaris, Piptatherum, Psathyrostachys, Pseudoroegneria, Trisetum, Zoysia* |
| | D | *Andropogon, Astrebla, Bothriochloa, Bouteloua, Cenchrus, Chloris, Dichanthium, Pennisetum, Sorghastrum* |
| | E | *Axonopus, Brachiaria, Digitaria, Echinochloa, Eleusine, Melinis, Panicum, Paspalum, Pennisetum glaucum, Setaria, Urochloa* |
| 2 (cereals) | A | *Avena, Hordeum, Secale, XTriticosecale, Triticum* |
| | B | *Oryza, Sorghum, Zea* |
| 3 (small legumes) | A | *Aeschynomene, Alysicarpus, Anthyllis, Astragalus, Centrosema, Chamaecrista, Securigera, Crotalaria, Desmodium, Galega, Hedysarum, Kummerowia, Lespedeza, Leucaena, Lotus, Macroptilium, Macrotyloma, Medicago, Melilotus, Onobrychis, Ornithopus, Securigera, Trifolium, Trigonella* |
| 4 (pulses) | A | *Vicia (small)* |
| | B | *Cajanus, Cicer, Lathyrus, Lens, Mucuna, Phaseolus coccineus, Pisum, Psophocarpus, Vicia (large)* |
| | C | *Arachis, Cyamopsis, Glycine, Lablab, Lupinus, Phaseolus, Pueraria, Vigna* |
| 5 (other agricultural species) | A | *Beta, Brassica, Dichondra, Hibiscus, Linum, Plantago,Sinapis,Spergula,Raphanus* |
| | B | *Cannabis, Carthamus, Fagopyrum, Helianthus* |
| 6 (vegetables, including fruits, spices and condiments) | A | *Achillea, Anethum, Anthriscus, Apium, Arctium, Atriplex, Atropa, Camelina, Campanula, Carum, Chrysanthemum, Glebionis, Cichorium, Claytonia, Cuminum, Daucus, Eruca, Fragaria, Lactuca, Lepidium, Solanum,Solanum hybrids, Mamubium, Matricaria, Melissa, Mentha, Nasturtium, Nicotiana, Ocimum, Oenothera, Origanum, Papaver, Petroselinum, Phacelia, Physalis, Pimpinella, Portulaca, Rheum, Rosmarinus, Rumex, Satureja, Sesamum, Stylosanthes, Taraxacum, Thymus, Valerianella* |
| | B | *Allium, Asparagus, Capsicum, Corchorus, Coriandrum, Cynara, Foeniculum, Pastinaca, Raphanus, Sanquisorba, Scorzonera, Solanum, Spinacia, Tragopogon* |
| | C | *Abelmoschus, Borago, Cucumis, Cucurbita, Cucurbita hybrids, Citrullus, Gossypium, Ipomoea, Lagenaria, Luffa, Momordica, Ricinus, Tetragonia,* |
| 7 (forest species) | See Table 2A, Part 2 | |
| 8 (flower species) | See Table 2A, Part 3 | |

**Crop groups for viability tests using the Tetrazolium method**

| Crop groups | Species belonging to the following genera |
|---|---|
| 1 (grasses) | *Agropyron, Agrostis, Alopecurus, Anthoxanthum, Arrhenatherum, Brachiaria, Bromus, Chloris, Cynosurus, Dactylis, Deschampsia, Elymus, Elytrigia, Eragrostis, Festuca, Holcus, Lolium, Panicum, Pascopyrum, Phalaris, Phleum, Poa, Pseudoroegneria, Setaria, Trisetum* |
| 2 (cereals) | *Avena, Hordeum, Oryza, Secale, Sorghum, Triticum, XTriticosecale, Zea* |
| 3 (small legumes) | *Lotus, Medicago, Melilotus, Onobrychis, Ornithopus, Trifolium* |
| 4 (pulses) | - |
| 5 (other agricultural species) | *Helianthus, Brassica* |
| 6 (vegetables, including fruits, spices and condiments) | *Allium, Arctium, Cucumis,Lactuca, Solanum, Solanum hybrids, Ocimum* |
| 7 (forest species) | *Abies, Acer, Amorpha, Calocedrus, Carpinus, Chamaecyparis, Chameacyparis, Cornus, Corylus, Cotoneaster, Crataegus, Elaeagnus, Euonymus, Fagus, Fraxinus, Ginkgo, Ilex, Juniperus, Koelreuteria, Ligustrum, Liriodendron, Berberis, Malus, Malva, Pinus, Prunus, Pseudotsuga, Pyrus, Rosa, Styphnolobium, Sorbus, Taxodium, Taxus, Tilia, Viburnum* |
| 8 (flower species) | - |

**Benefits of ISTA Accreditation**

Seed producers who want to have their seed tested have to be sure that the results produced are reliable and reflect the true quality of the seed to be sold.

These results are influenced by many factors, such as competence of analysts, use of appropriate equipment, use of validated methods, accurate recording and reporting, etc. The factors that influence the test results are subjected to assessment to verify if these criteria are met. The criteria are formulated in the ISTA Accreditation Standard, which is based on the internationally agreed generic accreditation standard for testing and calibration laboratories standard. Hence, seed producers themselves may accredit their laboratory to ensure that in-house seed testing is done correctly.

Accredited laboratories are authorized to issue ISTA Certificates needed by seed producers to ship seed into various countries; the risk to ship seeds with faulty results on the test report shall be minimized, especially when seed lots are shipped overseas. Hence, using an accredited laboratory for testing of seed helps to increase acceptance of seed lots and thus reduces costs. In some countries, import of seed is only permitted if an ISTA Certificate accompanies the seed lot. ISTA Accreditation programme is internationally recognised and the work is endorsed by international organisations such as Food and Agriculture Organization (FAO), International Union for the Protection of New Varieties of Plants (UPOV) and World Trade Organization (WTO). The seed scheme of the Organisation for Economic Co-operation and Development (OECD) refers to the ISTA Rules. The ISTA Rules are applied by all countries which adhere to the OECD certification seed scheme, including individual European Union (EU) countries and the EU as a whole. International Seed Federation (ISF) rules for trade refer to the ISTA Certificates for seed analysis as required trading documents.

To realize the quality of the purchased seed is lower than expected is costly and sometimes even too late to do something about it. Hence, seed buyers are interested in buying seed of high quality, which is to be proofed through testing the seed lots' quality in a seed-testing laboratory. Test reports, e.g. ISTA Certificates issued by accredited laboratories provide the seed buyer with confidence that the results on the test report correspond to the quality of the seed lot.

**Accreditation is beneficial to the seed seller, buyer and to the laboratory**

**For the seed seller:**

- Issuance of ISTA Certificates and provide opportunity for the growth of seed business internationally.
- Reduce costs by using an ISTA-accredited laboratory to increase acceptance of seed lots.
- Build on Accreditation to promote your business successfully with your government, customers and stakeholders.

**For the seed buyer:**

- Expect certainty that the results on the test report correspond to the quality of the seed lot

- Abide by the law of several countries where import of seed is only permitted if the seed lot is accompanied by an ISTA Certificate.

**For the laboratory:**

- Evaluation of work performance of the lab through Proficiency Test programme
- Receives formal recognition and assurance that the lab is performing the work correctly
- Boost up the confidence to produce reliable results
- Seek performance and remain up to date with latest testing methods.
- Develop internal pride and staff motivation.

ISTA Accreditation gives formal recognition to a laboratory that it is technically competent to test seed using the ISTA methods and producing reliable results. The laboratory receives assurance that its work is performed correctly and appropriately through the independent technical evaluation of a laboratory's performance. During the on-site assessment, various aspects of the laboratory's work are looked at, improvement potential and non-conformities are identified. As accreditation is becoming more and more accepted by authorities, customers and stakeholder to be an efficient tool to evaluate a laboratory's testing performance, accreditation is in fact an important marketing tool

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# SEED DETERIORATION: PHYSIOLOGICAL ASPECTS

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## Seed Deterioration

Seed deterioration is an undesirable and detrimental attribute of agriculture. Destructive condition of the seeds due to various reasons results in seed deterioration. Seed deterioration can be defined as the loss of quality, viability and vigor either due to ageing or effect of adverse environmental factors**.** The rate of deterioration rapidly increases with increase in either seed moisture content or temperature of storage (Ellis *etal.*1985). Losses in seed quality occur during field weathering, harvesting and storage. Rapid deterioration occurs due to the environmental conditions which makes it very difficult to maintain its viability during storage. Several environmental factors contribute to seed deterioration and these conditions are very difficult to maintain viability during storage. Quality of the seed depends upon initial seed quality, temperature, moisture content and seed handling.

## Factors of Seed Deterioration

### *I. Physiological parameters*

**1. Germination percentage:** A germination test was carried out following ISTA (2011), with four replicates of 100 seeds of each working sample. Final count was taken based on number of normal seedlings in each replication and the germination was calculated and expressed in percentage

**2. Moisture content:** Seed moisture content was determined using the low constant temperature oven method (ISTA, 2011) in two replicates of five grams seed material. The powdered seed material was placed in a weighed metal cup, and after removing the lid, moisture cups were placed in hot air oven maintained at 103 ± 2° C for 16 ± 1 h and the contents were allowed to dry. Moisture content was calculated as a percentage of fresh weight. Deteriorative reactions occur more readily in seeds at higher moisture content and subsequently, this condition constitute hazard to the longevity of seed survival (Vashisth and Nagarajan, 2009). Seeds stored at high moisture content demonstrate increased respiration, heating, and fungal invasion resulting in reduced seed vigor and viability. After physiological maturity the rate of seed quality loss depends on the degree of unfavorable environmental conditions surrounding the seed. The moisture content of seed during storage is the most persuasive factor affecting the longevity. Storing seeds at high moisture content enhances the risk of quick deterioration in shorter time. The lower the moisture content, the longer seeds can be stored provided that the moisture level can be controlled all through the storage period.

**3. Seed vigour:** 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 growth. The need to be able to identify seed lots having low vigour and therefore likely to emerge poorly has led to seed deterioration.

**4.Vigour index:** Vigour Index-i was calculated as the product of seedling vigour (root and shoot length) and germination percentage, vigour index –ii was calculated by gernmination % multiply by root shoot dry wt.(Abdul-Baki and Anderson, 1973). The viability of the seed accession is a measure of how many seeds are alive and could develop into plants. Disturbance in this index and atmospheric condition leads to seed deterioration.

**5. Imbibition percentage**- Imbibition is a special type of diffusion when water is absorbed by solids-colloids causing an enormous increase in volume. Examples include the absorption of water by seeds. If it were not for the pressure due to imbibition, seedlings would not have been able to emerge out of soil into the open; they probably would not have been able to establish.

**6**. **Seed protein**- Protein content can be determined by estimating total amount of nitrogen present in the seed and then multiplying it by 6.25(conversion factor). Total nitrogen can be estimated by Kjeldahl method (1983).

**7. Enzymes activity**- The enzymes play an important role in the progress of seed deterioration and changes in their activity can be an indication of quality loss (Copeland and McDonald, 1995). All enzyme activity is positively correlated with germination of seed. As ageing progress germination also decreases and enzyme activity also decreases which shows significant deterioration in both accelerated as well as in natural aged seed lot. All seeds undergo aging process during long-term storage which leads to deterioration in seed quality, especially in the humid conditions.

**8. Temperature-** The losses are worsened if seeds are stored at high temperatures and high relative humidity conditions (Mosavi Nik et al., 2011). High temperature hastened the rate of these biochemical processes triggering more rapid deterioration that resulted in rapid losses in seed having high moisture content (Shelar et al., 2008). Seeds sensitivity to high temperatures is strongly dependent on their water content, loss of viability being quicker with increasing moisture content (Kibinza et al., 2006).

**9. Genotypic Factors**- Some types of seeds are inherently long lived; others are short lived, while others have an intermediate life span owing to their differences on genetic makeup. One cannot generalize too broadly about seeds: cottonseed can be stored or kept for 2 or even 3 years while soybeans often deteriorate before the first planting season after harvest.

**10**. **Seed maturation-** Deterioration is at its lowest level at the time of seed maturation. It is generally not realized, seeds usually reach maturity long before normal harvest and at relatively high moisture contents - 30 to 45%.

Once the peak of maturation is attained, the seeds having reached maximum dry weight, vigor and viability, there is only one direction to go, downhill. There can be considerable deterioration before harvest.

***II. Mechanical Parameters***

**1**. **Pre-harvest environmental conditions**- Field condition during seed development and maturation have an important influence on the subsequent characteristics of the seeds: prevalence and persistence of hard seededness and dormancy, extent of mechanical damage, viability, vigour, test weight, appearance, and storability. Dry-hot weather leads to a rapid loss in seed moisture, small seed size, low test weight and more mechanical damage during harvest. When these are mixed together - the result is low quality seed. While this situation does not always occur- cool, dry weather during the maturation period minimizes deterioration.

**2**. **Harvest and Post-harvest Conditions**- Mechanical damage is one of the major causes of seed deterioration during storage. The quality of the seed is highly affected by harvesting and handling methods. Harvest and post-harvest deterioration comprises threshing, processing machinery, seed collection, handling, transporting and drying. Very dry seeds are prone to mechanical damage and injuries. Such damage may result in physical damage or fracturing of essential seed parts; broken seed coats permit early entry and easy access for microflora, make the seed vulnerable to fungal attack and reduce storage potential (Shelar, 2008). In its severest form, physical seed damage is exhibited by splitting of the cotyledon, shattered and broken seeds. Large seeded varieties are more sensitive to mechanical damage than small seeds.

**3**. **Storage-** Storability of seeds is mainly a genetically regulated character and is influenced by quality of the seed at the time of storage, pre-storage history of seed (environmental factors during pre and post-harvest stages), moisture content of seed or ambient relative humidity, temperature of storage environment, duration of storage and biotic agents (Shelar et al., 2008; Baleševiæ-Tubic et al., 2005; Khatun et al., 2009; Biabani et al., 2011). Seed moisture content is the most important determinant of longevity in storage. These environmental conditions are very difficult to maintain during storage. The seed storage environment highly influences the period of seed survival. Storage of one kind of seed at 12% moisture may be feasible, while for a different species 12% moisture might be too high.

**Physiological Seed Deterioration Detection Tests**

**1**. **Controlled Deterioration Test (CDT)**

This test, like accelerated ageing, increases the rate at which seeds age. In this case however, the degree of ageing sustained more closely controlled by raising the moisture content of all seed lots under test to the same level, usually 20%, before they are aged at 45$^0$C ( Matthews and Powell, 1981). The seed moisture

content is raised by placing seeds of known moisture content on moist germination papers and allowing them to imbibe until they reach the required moisture content. The achievement of this raised moisture content is checked by frequent re- weighing. The seeds are then sealed into aluminum foil packets, 700 gauge polythene packets or glass vials. Air should as far as possible be excluded from the packets and if glass vials are used there should be no air space remaining above the seeds. The packets or vials are then held overnight at $10^0$C for the moisture to equilibrate throughout the seed. An alternative method of raising seed moisture content is to add the required amount of water to the seeds in the vials or packets and hold the seed at $10^0$C for 24 - 48 hours. Although this gives a similar overall moisture content, we have found that the variation in the moisture content of individual seeds tends to be greater by this method. After the period of moisture equilibration, the seeds in the packets are placed at $45^0$C for 24 hours after which a germination test is set up. In this case, any seed that produces radical is considered to be germinated. The results of the controlled deterioration (CD) test co-relate well with both the field emergence and the storage potential of many vegetable seeds ( Matthews,1980). Thus seeds that have a high germination after CD are those which emerge and store well. Seed lots having a low germination after CD are however low vigour, lots with poor emergence and storage potential. They have undergone physiological ageing and lie towards the end of the slow decline so that further ageing during CD results in a fall in germination.

**2. Accelerated ageing Test**

The accelerated ageing test involves holding seeds at a high humidity (90 -100% RH) at $40^0$C. Temperature for varying lengths of time depending on seed type. This period of rapid ageing is then followed by a germination test. Seeds with high germination are considered high vigour seeds and those with poor germination, low vigour seeds which result in seed deterioration ( Agrawal and Dadlani, 2010).

**3. Tetrazolium Test (TZ)**

The tetrazolium test is essentially an assessment of dehydrogenase activity. This group of enzymes reduces colorless solution of 2, 3, 5 – triphenyl tetrazolium chloride to an insoluble red compound called formazan. Though commonly used as a viability test, this can also assess the seed vigour by a colorimetric estimation of color intensity kittock and law (1968),ISTA(2003b) described a method of estimating seed vigour on the basis of color intensity of stained seed embryo. This can differentiate the relative vigour levels between different seed lots.

**4. Electrical Conductivity Test**

This test is based on the leakage of solutes that occurs from all seeds into water. These solutes include sugars, amino acids and most importantly for the test electrolytes. This means that the incidence of leakage can be detected by measurement of the electrical conductivity of the seeds soak water. Low leakage and therefore low conductivity was associated with seeds that emerged well, that is high vigour, whereas low vigour seeds with poor emergence had high levels of leakage and conductivity (Mathews and Bradnock, 1967,ISTA (1995). The test will identify where solute leakage occurs as a result of decreased membrane integrity and the death of tissue during the ageing of the seed. The Electrical Conductivity is inversely proportional to the vigour of the seed.

**5. Cold Test**

The cold test has been most commonly applied to maize although it is also useful for cotton, sorghum and soybean (Fiala, 1981). It provides the stresses of low temperature, high soil moistures and the presence of soil-borne pathogens. The test involves planting at least four replicates of 50 seeds in unsterilized soil. The test is frequently carried in the boxes or trays as described below, although a rolled paper towel method has also been developed (Fiala, 1981) The results of a cold test are usually expressed as the percentage of normal seedling produced.

**CONCLUSION**

Seed deterioration can be defined as the loss of quality, viability and vigour either due to ageing or effect of adverse environmental factors**.** Several environmental factors contribute to seed deterioration. Quality of the seed depends upon initial seed quality, temperature, moisture content and seed handling. Important factors involved to seed deterioration are storage, moisture content, temperature, germination, seed vigour, viability, ion leakage, enzymatic activity etc. Various physiological tests can be conducted to check the deterioration in seeds.

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# SEED QUALITY ENHANCEMENT - PRINCIPLES AND PROCEDURES

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Seed enhancement is relatively a new area of seed technology, and considerable research has been conducted over the past 15 to 20 years. Seed enhancement may be defined as post harvest treatments that improve germination or seedling growth or facilitate the delivery of seeds and other materials required at time of sowing (Taylor et al 1998). This definition includes three methods: pre sowing hydration treatment (priming), coating (pelleting) technologies and seed conditioning.

The so called modern seed enhancement technologies have historical basis dating back hundreds to thousands of years. The principles and concepts have changed little with time. Seed coating was being practiced by ancient Chinese farmers who coated the seeds of rice with mud balls to anchor the seeds in water saturated soils (Ni 1977); the Greeks who presoaked the Cucumber seeds in milk or water to make them germinate quicker (Evenari 1980). Keeping the seeds in moist cloth or soaking in water prior to sowing was a common practice among the farmers of India from time immemorial. Inspire of all these insight into the ancient farming technology the physiological seed treatment have not been widely exploited in contrast to pervasive uptake of crop protectants applied to seeds.

## Seed Hydration

The objective of seed hydration is to increase the percentage and rate of germination ,expand the range of temperatures over which the seed will germinate and increase the uniformity of stand establishment. To accomplish this seed must be hydrated in some way at a moisture level sufficient to initiate the early events of germination (phase II of imbibitions) but not sufficient to permit radicle protrusion (phase III) The general approaches to hydration can be pre hydration ,priming and solid matrix priming.

## Prehydration

Soaking seeds in water prior to sowing enhances germination and seedling growth by controlling the imbibition conditions and reducing the vagaries of adverse weather and soil conditions (Bradford 1986). Instead of soaking the seeds directly in water the modern approach is the seeds are often pre germinated in gels at 20 0C and planted : a process known as fluid drilling (Gray 1981) .The gel protect the seeds as well as provide moisture. Examples of gel used are hydroxyethyl cellulose ,magnesium silicate and polyacrylamide (Taylor and Harman1990).The gel can contain activated carbon ,added nutrients and pesticides further it improve seedling performance .Pre hydration also can be followed by drying if seeds are not germinated (hydration dehydration)to facilitate

subsequent handling and storage. This method is similar to the technology known as "on farm seed hydration" method which has become popular in the rain fed agriculture. The physiological mechanism that result in improved performance is considered similar to those involved in seed priming (Karssen and Weges1987).

**Seed Priming**

Priming can be defined as controlling the hydration level within seeds so that the metabolic activity necessary for germination can occur but radicle emergence is prevented. This is because different physiological activities within the seed occur at different moisture levels (Leopold and Vertucci 1989, Taylor 1997). In other words the water uptake follows a triphasic pattern with a rapid uptake phase known as imbibition phase (phase I ) followed by a lag period (phase II) and then a second increase in water uptake associated with seedling growth or radicle emergence (phase III ). By limiting the water content, all the metabolic steps necessary for germination can occur without the irreversible act of radicle emergence. Prior to emergence the seed is considered desiccation tolerant and so the primed seed can be dried and stored before sowing

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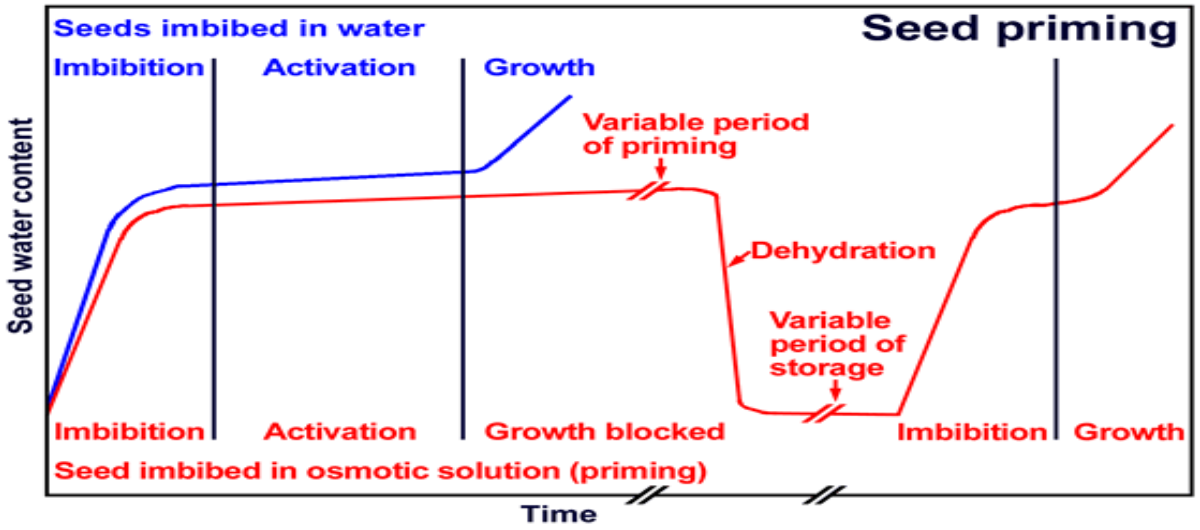



© 2006 Gerhard Leubner - The Seed Biology Place - http://www.seedbiology.de - Redrawn/modified from: Bradford KJ, Bewley JD (2002). Seeds: Biology, Technology and Role in Agriculture. Chapter 9, pp. 210-239. In: Plants, Genes and Crop Biotechnology (eds Chrispeels MJ, Sadava DE), Jones and Bartlett, Boston.

Several methods have been described to regulate the water availability to the seeds both at liquid and vapor phase (Khan 1992). Seed priming methods can be grouped into two categories depending upon whether the water uptake is controlled or no controlled. No controlled water uptake includes those methods in which water is freely available and is governed by the seed to the water. The seeds may be soaked, or kept on moist blotters. Here, since the availability of water is mostly controlled the seeds will ultimately enter the 3rd phase. Therefore, limiting the duration of imbibitions at proper temperature must arrest the process.

In controlled water uptake, water availability or water potential is regulated thus preventing the seeds to enter phase III stage. There are three methods to limit water uptake: priming with solution, priming with solid particulate technique and drum priming by controlled hydration. Priming with solutions relies on the controlled hydration of water uptake by controlling water potential of the priming media throughout the period of priming. There are various inorganic salts, manitol / glycerol which when added to water decreases the osmotic potential.
Solid particulate system is also used to increase seed moisture in a controlled system. Water is added to the solid carrier and the seeds take up water from them in a controlled manner till equilibrium occurs. The water potential of the solid particulate environment is determined by the physical and chemical properties of the solid carrier. On a commercial scale, controlled hydration by drum priming elevate the seeds to a desired moisture level by applying given amount of water at specific time (Rowse,1996). Another drum priming system controls seed hydration by the time interval and volume of water application Warrer and Bennett (1997). The total water required is divided into different intervals and injected to the system. The prior knowledge of the water imbibitions capacity of the seed should be known in advance for this technique.

The benefits of seed priming technologies have been well documented in the review articles (Bradford 1986: Khan 1982: Taylor et al 1998.). However for practical purpose seeds are primed for the following reasons:

1. To. decrease the time necessary for germination and subsequent emergence to occur.
2. To improve stand uniformity in order to facilitate production management and enhance uniformity at harvest.
3. To overcome or alleviate phytochrome induced dormancy in specific species.

One of the primary benefit of priming has been to increase the rate of germination at any particular temperature. In practical primed seeds emerge from the soil faster and often more uniform than non-primed seeds. Priming accomplishes this important step in field by shortening the lag period in phase II of the germination process. Since the seed have already gone through this phase during priming germination time requirement can be reduced to approximately *50 per cent* upon subsequent dehydration. The increase in emergence speed and field uniformity demonstrated with primed seeds have many practical benefits. Emergence occur before soil crusting; seedling can compete more effectively with weeds; increased control can be exercised over water dosage and scheduling or water stress under rainfed conditions of sowing (Harris 1992).
Another important benefit of priming has been the germination of seeds under sub optimum temperature. (Valdese and Bradford 1987; Ellis and Butcher 1998). From a practical stand point priming enable seeds of several species to germinate

and emerge at sub optimal temperatures. Examples are available in almost all temperate vegetables like tomato pepper and lettuce. Our own studies have shown that hydration of seeds for 24hrs and then drying back to original moisture content improved total and speed of emergence in winter sown maize and sunflower.

Lastly priming has been commercially used to eliminate or greatly reduce the amount of seed borne fungi and bacteria. Organism such as *Xanthomonas compestris* in *Brassica* seeds and *Septoria* in celery has been eliminated within seed lots by priming. The mechanism responsible for eradication may be linked to the water potential that seeds are exposed during priming, differential sensitively to priming salts and oxygen concentration.

**Osmopriming**

Commercial development of germination enhancing treatments is of recent origin. The first report is of Hydecker et al 1973 of a method of incubating seeds on a blotting paper wetted with polyethylene glycol (PEG). Later on Darley and Salter 1976; Bijalski et al 1989 described a method of aerated PEG priming which enabled the commercial application of the technique. Cantiliffe 1981used solutions of inorganic salts to create the required osmotic potential or other solutions like manitol (Gerorghiou et al 1987.When incubated in PEG or salt solutions the seeds does not imbibe water to the same degree as in pure water. (Fig) when water is freely available seeds generally imbibe until they reach a moisture level of around 50%on a fresh weight basis. Seeds incubated in PEG solutions the moisture content of seed is raised to around 40%which is often PEG concentration and species specific .in general water potential between –1.0 and –1.5 Mp are used the water potential of the seed will be equivalent to the solution surrounding it at the end of the treatment because of the low moisture content, and the metabolic process in the seed proceed at lower rate than when primed in water and so the emergence of the radicle is prevented. Because of this PEG or other osmotic priming can proceeded for a longer duration. Some of the common osmotic priming agents used along with the species are given in table 1.

**Table1:** Selected species and osmotica used for seed priming

| **Species** | **Osmoticum** |
|---|---|
| Asparagus | PEG, NANO3, saline water |
| Sugar beet | PEG, MgSo4, NaCL |
| Broccoli | PEG Cabbage PEG |
| Carrot | PEG, Sodiumpolypropionate, KH2PO4, Glycerol, KNO3 |
| Celery | PEG Sodiumpolypropionate, KH2PO4, Glycerol, KNO3 |
| Cucumber | Mannitol, NaCL |
| Eggplant | Mannitol |
| Leek | PEG |

| | |
|---|---|
| Lettuce | PEG |
| Pepper | KH2PO4, KNO3+KH2PO4, NACl, CaCl, Na2HPO |
| Sorghum | PEG, KNO3, K2HPO4 |
| Soybean | PEG, Mannitol, PEG+GA3, Sodiumpolypropionate |
| Spinach | Sodiumpolypropionate |
| Sweet corn | PEG, Sodium hypo chloride |
| Tomato | PEG, Sodiumpolypropionate, KH2PO4, Glycerol |

Compared to PEG salt solutions are easy to use and also economical though PEG has the advantage, as it is chemically inert and the large molecular size prevent penetration into the seed. In osmopriming osmotic agents could be combined with seed protectants and growth regulators to improve the effect of priming .Peg combined with GA3 could improve germination under stress conditions like sub optimal temperatures and soil crusting in soybean Lorenz1988) sweet corn (Vari et al 2000). For commercial priming with PEG, vessels with bubble columns and stirred bioreactor are being used (Gray et al 1992) under license in UK
A refinement of PEG priming is membrane priming described by Rowse et al 2001) In this seeds are separate from the solution by a membrane which is contained within the walls of a double walled cylithe advantages are better aeration reduction in use of Peg and possibility of priming small amount of seeds at atime. This method was found beneficial to seeds of verbena and pansy (Rowse 2001).

**Solid Matrix Priming**

Solid matrix priming consists of mixing seeds with an inorganic or organic carrier and water for a specified period of time .The moisture content of the matrix is brought to a level just below what is required for radicle protrusion. Seed water potential is regulated by the matrix potential of the carrier till the equilibrium is reached. This controlled hydration allows specific quantity of water directly in contact with the seeds in order to achieve the elevated moisture content. Different carrier materials have been used in SMP priming (Table 2). The ideal characteristics are i) no phytotoxicity, ii) high water holding capacity iii) remain friable at different moisture level iv) easy to remove after treatment.
The advantage of SMP are; large quantity of seeds can be treated at a time with less amount of priming solution and the imbibitional injury to seed can be controlled. Also during the process addition of fungicides and growth promoters can be incorporated.

**Table: 2** Selected species and SMP materials used

| **Species** | **Solid Material** |
|---|---|
| Beet root | Mcrocel E+fungicide, Vermiculate#5,Agro-lig |
| Broccoli | Vermiculate#5 |
| Carrot | Mcrocel E, Vermiculate#5,Leonardite shale |

| | |
|---|---|
| Celery | Calcinated clay +NAOCL |
| Onion | Leonardite shale |
| Pea | Leonardite shale+ trichoderma |
| Sweet corn | Leonardite shale, calcinated clay +NAOC |
| Tomato | Leonardite shale+trichoderma, Spagnum mos+tricho |

**Drum priming**

The drum priming method developed by Rowse involves the hydration of seeds over a period of 24-48 hrs in a drum revolving a 1-2 cm /sec.here mixing of seed has to be very uniform .the degree of hydration is determined by a simple calibration test for each lot A measured volume of water is injected during each cycle as regulated by a timer attached This technique is commercially under license in UK (Gray 1996)

**Seed Pelleting**

Seed pelleting refers to the addition of inert materials added to seed to change the size and shape resulting in substantial weight increase for improved plantability. Small and irregularly shaped seeds are made larger and round shaped precise seed placement is of great advantage as uniform bulk development is assured with equal distance planting. The pelleting materials may contain polymers, pesticides biological identifying colorants dyes or other additives. There are two components to a seed pellet bulking (coating) material and a binder. The bulking materials can be a mixture of several different minerals and / or organic substance or a single component. The coating materials change the shape .size and weight of seeds. It is important that the coating material should have uniformity of particle size distribution, availability, and lack *of* phytotoxicity._The other component of the pellet the binder, holds the coating material together. The concentration *of* the binder is critical because too much binder hinder germination and *too* little result in chipping and cracking in the pellet box. Many different compounds have been used as binders, including various starches, sugars, gum arabic, clay, cellulose, vinyl polymers Halmer (1987).

Seeds of various sizes and shapes are commercially pelleted from fairly large seeds like onion and tomato to very small seeds like Begonia. In onion seed can increase in weight by 6-fold due to pelleting while in Begonia it is l00-fold. Seed weight for raw begonia is 88,000 seed gr-l and after pelleting it is 857 seeds g-l (Seed Dynamics)

The physical nature of the filler directly affect the pellet weight or density and is determined by the sowing requirement. Low density pellets are used for the seedling equipment in green house planting and high density for large scale high speed field planting. For example lettuce pellet with same volume formulated for different densities are available. Increased competition in the pelleted seed market has fastened the development of more effective pellet with greater

capacities and wider planting characteristics. Improvement in the last 10 years includes increased oxygen penetration availability, wider pellet density range, pellet loading, better field viability. Pellet may act as barrier to oxygen diffusion thus affecting germination. This problem has been overcome by addition of oxygen liberating compounds into pellet or modification of pellet breakdown after sowing or splitting pellet technology.

Pellet loading: The act of applying a plant protectant in a band within the pellet is known as 'pellet loading'. The pellet either act to 'dilute' the negative impact of a plant protectant or act as a barrier to prevent direct contact as same plant protectant if applied directly to seed are deleterious can be carried in the pellet. Active product can thus be loaded into the seed while minimizing adverse seed germination effects. The total amount of 'toxicants' applied per acre is less than direct application to soil. Thus chemical loading of the pellet provides a means to treat the seed directly and considered better than the seed directly and considered better than other application such as furrow, foliar, or broadcast because of the following reason:

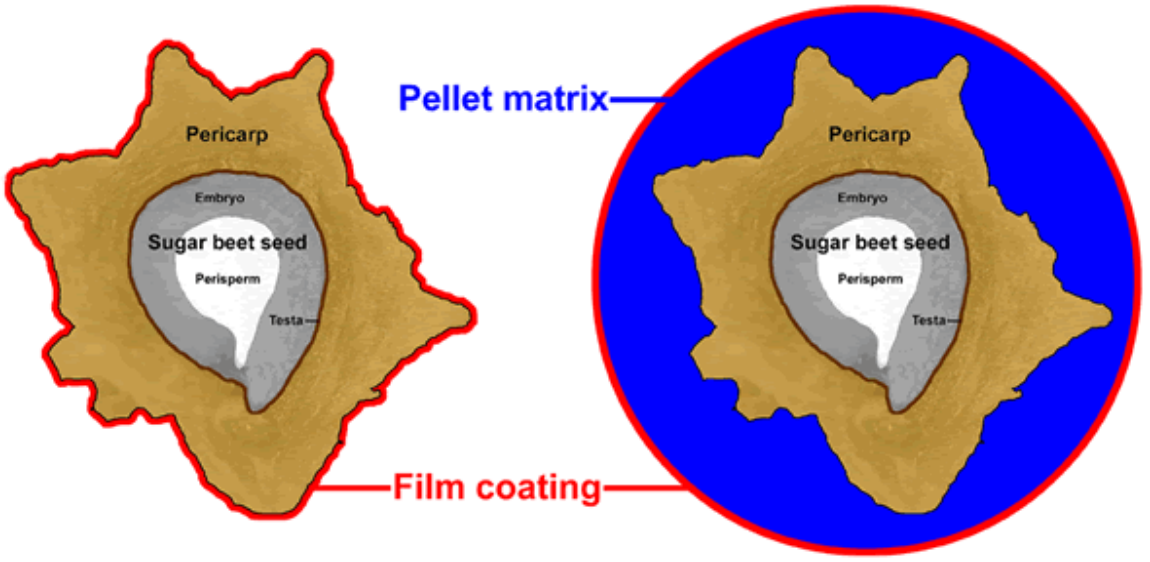
i)Precise placement on the target
ii)Minimum toxicant used
iii)Minimum exposure to workers and wild life

**Seed Coating:**

The seed coating substance such as fungicides micro-organisms, micronutrients etc are added to the seeds without observing the shape of the seeds. The major benefits of this technology is that enhancing material is placed directly to the seed and smaller amount of chemicals are needed as compared to broadcasting. Film coating is the most commonly used technology in which precise amount of active ingredients along with liquid material is added directly to the surface of the seeds which may increase the seed weight by 10%. Since the coating in this multiple coating is possible including coloring material.

Some of the commonly available pelleting technologies and their advantages are given below.

**Incotec:** Pelleting to increase the size and density of seeds like Begonia and Lettuce for precision planting. Approved fungicides and insecticides are incorporated to pellets and are available at field establishment of seedlings.






**Split Pill**: This is a unique feature in which the coating material allows a free and quick access of moisture and oxygen to the seed. The pellet split open and establish.

**Split pill Light:** A light weight pellet suitable for sowing under fluctuating moisture levels.

**Split pill special :** Pellets are available with thirum soak treatment for celery seeds.

**Split pill Thermocure:** Split pill in combination with thermocure treatment. The thermocure provide maximum germination under stress conditions. Like high low temperature etc.

**Seed Coating Machinery:**

Rotary type seed coater has many advantage over traditional seed treatment equipments such as

- Precise material application
- Separate delivery system for coating and pesticides.
- Efficient usage of coating.
- Electronic digital scale.
- Capable of film coating of any type of seed.
- Capable of pelleting with power filling and decoating.
- Easy to operate and maintain.

A film coating using thin layer of polymer over the seeds without significantly increasing the size of the seed and adheres the fungicides preventing it from dusting off. The film is readily soluble in water and does not impede germination. The benefits are-

- Application of accurate dose of chemicals.
- Sowing accuracy due to coloring applied through coating.
- Precise placement and flow of seed.
- Reduces exposure to chemicals while handling.
- Provides effective strategy against infection by Botrytis Alli.
- Reduces effect of damping off and assist onion seedlings in soils with white rot.
- Enables application of trace elements to seed.

These seed quality enhancing technologies, priming, polluting and conditioning have been commercially exploited in the developed nations and almost *100 per cent* of the vegetable seeds marketed by seed companies are the product of these technology. Most of these technologies have been patented and are proprietary. However. simpler technology suitable for tropical and subtropical countries are available though their commercial acceptance is still at large. Single short duration soaking of seeds for 4-8 hours followed by light air drying improves overall germinability rapidity and synchrony of germination in non leguminous seeds (Basu 1994), For leguminous seeds moisture equilibration at 100% RH for 24 to 48 hrs, or matriconditioning with inorganic carriers like Microcel – Cel are recommended. Pre- storage dry -dressing of freshly harvested seeds using common bleaching powder @ 3g/Kg of seed or mixing with inert carrier like calcium carbonate containing very low concentration of iodine or alcohol would improve storability of many dry stored agriculture and horticulture crops. Mid storage hydration -dehydration treatment which include short term soaking - drying for low and medium vigour seeds and dipping drying were found effective in extending storability and subsequent field performance. Commercial application of seed priming in the semiarid (Rainfed) region in the tribal areas of M.P. have been successfully demonstrated in maize, chickpea, pearlmillet, sorghum and horse gram. With the availability of seed lots having 100 % viability through conditioning, with faster emergence through seed priming and uniform and precise population stand and disease control through pelleting, maximum crop yield could be attained especially for high cost low volume vegetable and hybrid seeds. Seed technology advancement have a major role to play to enhance agricultural production as land availability decreases and competitive demand for maximum production increases.

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# GLOBAL STATUS OF SEED INDUSTRY WITH SPECIAL REFERENCE TO INDIA

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The international seed industry is highly sophisticated. Global breeding programs integrate different sources of elite germplasm and biotech traits to produce new hybrids and varieties. These can be adapted to local conditions. Elite germplasm remains the foundation of plant breeding, but the new tool-box of biotechnology provides the means to characterize and use it more effectively. Breeding cycles can be reduced, allowing new products to be developed more quickly and with greater confidence.

## Value of the Global Seed Market

Global seed market is estimated in 2014 to be US $ 50 billion where as it was US $ 19 billion in 2005. So, in a span of about 9 years the industry value has increased to about 2.6 times. The share of North America and Europe in Global seed market is more than 50%.
It is conservatively estimated that the top 10 companies control 62% of the global seed market (Fig.1).

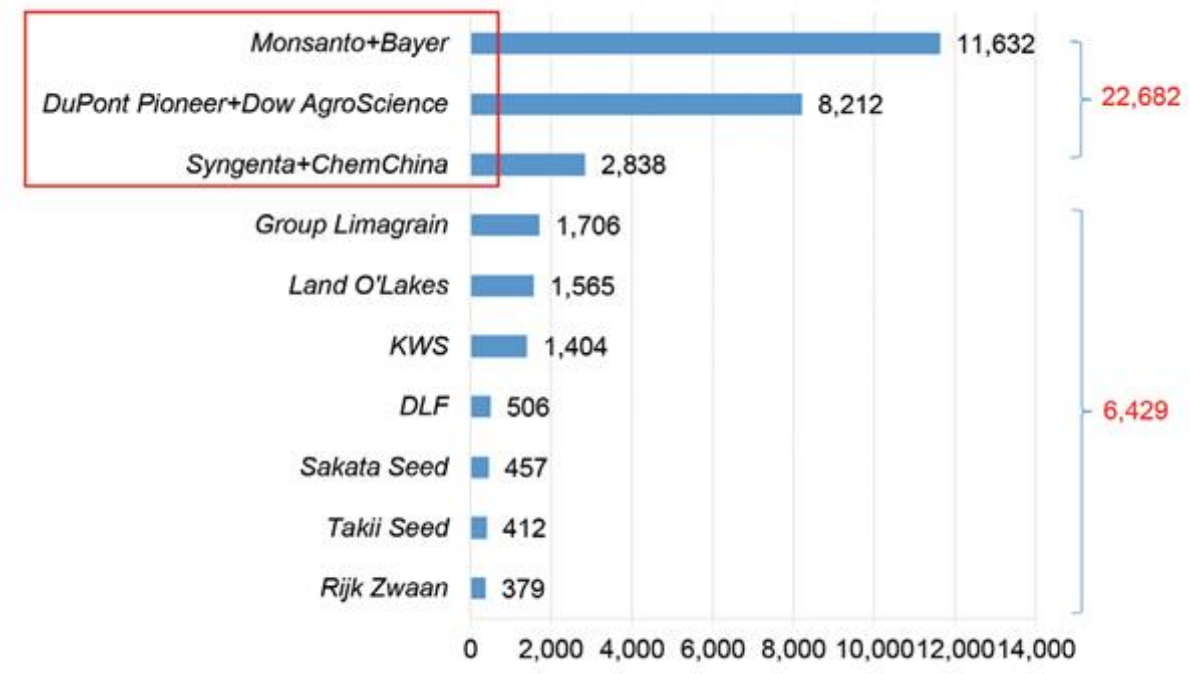


**Fig.1:** Turn Over of the top 10 seed companies (US $ million)

The top 6 players (After merger only 3 players) turnover is 3.6 times of the remaining 7 players.

**Market Share**

Today, the top 10 companies control more than half of the world's commercial seed sales. With a total worldwide market of approximately US$ 50 billion per annum, the commercial seed industry is relatively small as compared to pesticide market. The Global value of the Pesticide Industry is 207.5 billion in 2014. But, corporate control and ownership of seeds – the first link in the food chain – has far-reaching implications for global food security.

**Food Security**

With control of seeds and agricultural research held in fewer hands, the world's food supply is increasingly vulnerable to the whims of market maneuvers.

Corporations make decisions to support the bottom line and increase shareholder returns and not to insure food security. Ultimately, the growers may have fewer choices for variety. A study by the US Department of Agriculture concludes that reduced competition is associated with reduced Research and Development.

Despite seed industry claims to the contrary, concentration in the seed industry is resulting in less innovation and not more.

**Genetically Modified Seeds**

- Despite controversy, genetically modified (GM) seeds are gaining considerable market share.
- The market for biotech seed traits (herbicide tolerance and insect resistance) has shot up from $280 million in 1996 to $4,700 million in 2004, a 17-fold increase over the past nine years.
- In 2004, Pioneer/DuPont earned 50% of its seed revenues from varieties that included a genetically modified trait
- The growth in GM seed market share is remarkable, especially given that genetically modified seeds have been accepted in relatively few countries and continue to be steeped in controversy all over the world.

**Seed Industry in India**

It is a vibrant seed industry. Estimated Turnover of the seed industry in India is Rs. 258 billion in 2017. The seed companies could be grouped in the following:

- Private Seed Companies about 200 in no., out of which 25-35 co. may be having <150-400 crores turnover
- Public Sector state Seed Corporations- 26 in no.
- One Central Seed Corporations
- Seed Cooperatives and NGO

In India transgenic cotton has been commercialized in 2002. It took 6-7 years for conducting bio-safety and agronomic trial before the GEAC (Genetic Engineering Approval Committee) gave permission for commercialization. The

first three Bt cotton hybrids were commercialized on March, 26, 2002. It was from Maharashtra Hybrid Seed Company, a pioneer and visionary company. Today almost 95% of the cotton growing area in India is covered by Bt cotton. There are several events commercialized for cotton in India, which are given in the table:

**Table 1** Details of Bt events along with approval date and their sources

| **Event/Gene** | **Source** | **Date of Approval** |
|---|---|---|
| MON 531 (BG), cry 1Ac gene | Mahyco-Monsanto | 2002 |
| MON 15985 (BG II) {cry1Ac & cry 2 Ab genes} | Mahyco-Monsanto | 2006 |
| Event 1 {cry 1Ac gene} | IITKharagpur, commercialized by J K Agri-Genetics | 2006 |
| GFM Event {cry 1Ab & cry 1Ac genes} | Chinese gene commercialized by Nath seeds | 2006 |
| BNLA-601 {cry 1 Ac gene} | CICR (ICAR) & UAS Dharwad | 2008 |
| MLS-9124(synthetic cry 1Ac gene) | Metahelix Life Sciences , Bangalore | 2009 |

Out of the several events listed above the most popular and heavily used is the event from Mahyco-Monsanto.

**International Organization for Seed Quality Assurance**

There are three organizations are involved internationally, in addition to National organizations viz.,

- International Seed Testing Association (ISTA)
- American Official Seed Analyst (AOSA)
- Organization of Economic Cooperation and Development (OECD)

# PLANT GENETIC RESOURCES MANAGEMENT: NATIONAL GENEBANK

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National Bureau of Plant Genetic Resources (NBPGR) is the nodal institution at national level for management of PGR in India under the umbrella of the Indian Council of Agricultural Research, New Delhi. The Bureau after its creation in 1976 has developed a very strong Indian Plant Germplasm Management System which operates in a collaborative and partnership mode with other organizations. The system has contributed immensely toward safeguarding the indigenous crop genetic resources and introducing the useful PGR from other countries for enhancing the agricultural production and productivity in country. India being one of the gene-rich countries of the world faces a unique challenge of protecting its natural heritage and evolving suitable mutually beneficial strategies for germplasm exchange with other countries.

The National Genebank Network consists of the National Genebank at ICAR-NBPGR, New Delhi, which is primarily responsible for conservation of germplasm on long-term basis. The 10 regional stations NBPGR and facilities (Tabel.1), in different agro-climatic zones of the Country and the 59 National Active germplasm Sites (NAGS) (Fig.1) are the integral component of the Network. The NAGS are based at the premier institutes for specific crops or crop groups and are entrusted with the responsibility of multiplication, evaluation, conservation of active collections and their distribution to users both at national and international levels. Various other National institutes, All India Coordinated Crop Research Projects, State Agricultural Universities, International Agricultural Research Centers involved in conservation and use of PGR and other stakeholders are also effectively linked to the network.

## Plant Biodiversity

Indian subcontinent has a rich and varied heritage of biodiversity, encompassing a wide spectrum of habitats from tropical rainforests to alpine vegetation and from temperate forests to coastal wetland. It is one of the eight centers of origin (Vavilov, 1951) and is one of the 12 mega gene centers of the world. It possesses 11.9% of world flora, and about 33% of the country's recorded flora are endemic to the region and are concentrated mainly in the North-East, Western Ghats, North West Himalayas and the Andaman and Nicobar islands. Of the 49,219 higher plant species, 5,725 are endemic and belong to 141 genera under 47 families (Nayar, 1980). Of these 3,500 are found in the Himalayas and adjoining regions and 1,600 in the Western Ghats alone (Arora, 1991). Indian region is an important centre of origin and diversity of more than 20 major agri-horticultural crops including rice, beans, cotton, sugarcane, citrus, mango, banana, yams and several common vegetables and

popular spices. Rich diversity occurs for crop plants (166 species) and their wild and weedy relatives (324 species) along with around 1000 species of ethnobotanical interest (Arora 1991). Indian region too is a major center of domestication and diversity of crop plants (Zeven and de Wet, 1982; Arora, 1991). Indian region too is a major center of domestication and diversity of crop plants (Zeven and de Wet, 1982; Arora, 1991).

**Table 1: Establishment of Regional Stations/Facilities**

| Year | Regional Stations/ Facilities |
|---|---|
| 1960 | Shimla (Himachal Pradesh) |
| 1965 | Jodhpur (Rajasthan) |
| 1977 | Akola (Maharashtra) & Thrissur (Kerala) |
| 1978 | Shillong (Meghalaya) |
| 1985 | Bhowali (Uttarakhand) & Hyderabad (Telangana) |
| 1986 | Cuttack (Odisha) |
| 1988 | Ranchi (Jharkhand) & Srinagar (Jammu & Kashmir) |
| 1983 | National Genebank |
| 1986 | NFPTCR (Now known as TCCU) funded by DBT |
| 1996 | NCR on DNA Fingerprinting |
| 1997 | Agriculture Knowledge Management Unit (AKMU) |
| 1999 | Containment facility (CL-4) funded by DBT |
| 2003 | Experimental Farm at IARI |


**Fig:1** National Active Germplasm Sites

**National Genebank**

The major components of the National Genebank include the seed genebank, field genebank, cryo genebank and the *in vitro* genebank (Fig 2.) The *ex situ* seed genebank at NBPGR comprises 12 long-terms modules (total capacity: 1 million accessions) maintained at -18$^0$C for hosing the base collections. The active collections are distributed in 22 medium-term modules maintained at 4$^0$C for storing germplasm at active sites. At present, the genebank holds more than 4.34 lakhs accessions (Table. 2). The accessions held in the long-term storage for 10 or more years are monitored for their viability, seed quantity etc. as per the genebank standards. According to the monitoring results the samples/lots registering viability below the specified standards are sent for

regeneration at the NAGS . The cryobank facility in the National Genebank has accessions of varied germplasm of orthdox, intermediate and recalcitrant seed species and also of pollen samples. The *in vitro* genebank conserves various priority crops which are maintained under short- to medium-term storage periods. These include tuberous and bulbous crops, tropical fruits species and industrial crops, medicinal and aromatic plants species. The cultures are maintained at standardized temperatures and are sub-cultured after 4 to 24 months' intervals. There is a need to adopt complementary conservation strategies involving both *in situ* and *ex situ* approaches. For *in situ* conservation due attention is required to be given to genetically rich hotspots including all the stakeholders, including and expand the network of germplasm conservation by including all the stakeholders, including the communities. It is to be ensured that a set of the available PGR with associated database must be deposited as base collection with the genebank, and one set maintained as active collection with the accessible for plant improvement. There is an urgency to assess the germplasm collection in the genebank to understand the gaps and also identify the duplicates.

**Table 2:** Status of crop diversity of base collections conserved in the National GeneBank (as on 1st January, 2018)

| Crop/Crop Group | No. of acc. conserved | Crop/Crop Group | No. of acc. conserved |
|---|---|---|---|
| **Cereals** | **1,61,816** | **Forages** | **6,925** |
| Paddy | 1,07,226 | Oats | 1,374 |
| Wheat | 33,429 | Clover | 595 |
| Maize | 10,965 | Teff | 297 |
| Others | 10,196 | Marvel grass | 333 |
| | | Others | 4,326 |
| **Millets** | **58,443** | **Pseudo Cereals** | **7,295** |
| Sorghum | 25,813 | Amaranth | 5,953 |
| Pearl millet | 8,156 | Buckwheat | 998 |
| Minor millet | 24,474 | Others | 344 |
| **Legumes** | **65,675** | **Oilseeds** | **58,571** |
| Chickpea | 14,661 | | |
| Pigeonpea | 11,661 | Groundnut | 13,819 |
| Mung bean | 4,184 | Oilseed brassica | 11,308 |
| Pea | 4,172 | Safflower | 7,364 |
| Cowpea | 3,798 | Sesame | 10,148 |
| French Bean | 3,938 | Soybean | 4,042 |
| Clusterbean | 4,313 | Sunflower | 1,382 |
| Horsegram | 3,047 | Others | 10,508 |
| Ricebean | 2,154 | | |

| | | | |
|---|---|---|---|
| Others | 13,994 | | |
| **Vegetables** | **26,071** | **Fibre** | **15,573** |
| Tomato | 2,595 | | |
| Brinjal | 4,433 | Cotton | 10,004 |
| Chilli | 4,895 | Jute | 3,245 |
| Okra | 3,695 | Mesta | 2,000 |
| Onion | 1,128 | Others | 324 |
| Others | 9,325 | | |
| **Medicinal & Aromatic Plants & Narcotics** | **7,929** | **Ornamental** | **653** |
| Opium poppy | 431 | | |
| Ocimum | 590 | Marigold | 362 |
| Tobacco | 2,265 | Others | 291 |
| Others | 4,643 | | |
| **Agroforestry** | **1,642** | **Spices & Condiments** | **3,074** |
| Sesbania | 647 | Coriander | 1,048 |
| others | 995 | Fenugreek | 1,294 |
| | | Others | 732 |
| **Duplicate Safety Samples (Lentil, Pigeonpea)** | **10,235** | **Trail Material (Wheat, Barley)** | **10,771** |
| **Total** | **4,34,946*** | | |

*The figure includes 5,034 Released Varieties and 4,316 Genetic Stocks; regenerated accession not included.

No. of Crop Species conserved : 1,762 (Source: http://www.nbpgr.ernet.in)

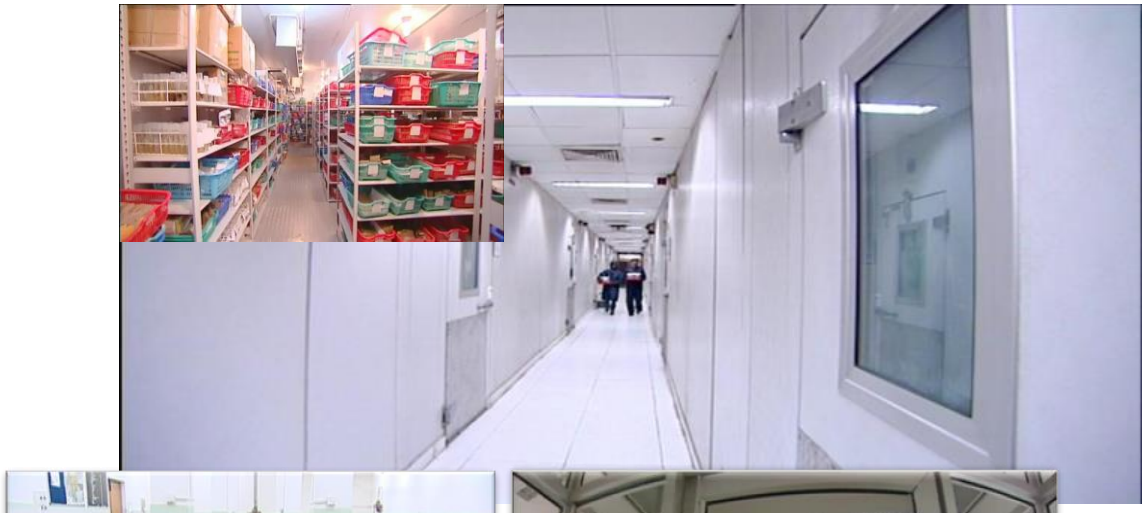
**Table 3:** Crop diversity conserved *in-vitro* in the National Genebank (As on 1st January 2018)

| **Crop / Crop Group** | **Present Status** | **Crop / Crop Group** | **Present Status** |
|---|---|---|---|
| **In vitro bank** | | **Cryobank** | |
| 1. Tropical fruits | 420 | 1. Recalcitrant | 0 |
| 2. Temperate and minor tropical fruits | 330 | 2. Intermediate | 6,585 |
| 3. Tuber crops | 518 | 3. Orthodox | 3,889 |
| 4. Bulbous crops | 171 | 4. Dormant bud (Mulberry) | 387 |
| 5. Medicinal & aromatic | 155 | 5. Pollen | 532 |

| | | | |
|---|---|---|---|
| plants | | | |
| 6. Spices and industrial crops | 227 | 6. DNA | 1428 |
| **TOTAL** | **1,821** | **TOTAL** | **12,821** |

(Source: http://www.nbpgr.ernet.in)

**Fig .2:** National Genebank: Serving and Conserving Plant Genetic Resources for Enhanced Food Security



Cryo Genebank


Invitro Genebank

Tissue Culture Repository

Field Genebank

**Seed Drying Facilities**

NGB-NBPGR: Walk-in seed drying room


NGB-NBPGR: Seed drying cabinet

**Packing and sealing**

**Hermatic sealing**

**Tri-layered aluminium foil pouch**

**Enhanced utilization of Plant Genetic Resources**

Germplasm utilization requires use of stable and heritable traits. Therefore, a network programme on multi-locational evaluation leading to utilization in crop improvement programmes of priority crops has been initiated. Evaluation of crops of agronomical, National Research Centres and All India Coordinated Research Centers and All India Coordinated Research Projects in being Carried out as detailed in Fig 3.

- I Year: Germplams accessions collected are multiplied and characterized for qualitative traits at a suitable location (NBPGR or relevant crop-based institutes/ PD/PC/NRC).
- II Year: The accessions are raised and evaluated for quantitative traits/ agronomic performance at relevant institutes (crop-based PD/PC/NRA). The seed produced will be divided into three parts required for (i) National Genebank, (ii) collaborative centre for further testing and use and (iii)

national supply (active germplasm) by NBPGR. First priority is to conserve the germplasm in National Genebank.

- III Year: Promising accession would be evaluated in National Germplams Nursery for agronomic traits by AICRP centres. This will be followed by evaluation for resistance/tolerance to biotic and abiotic stresses and quality traits. Germplasm will be screened under controlled environments as well as sick plots. The testing environments will be decided by the concerned PC/PD

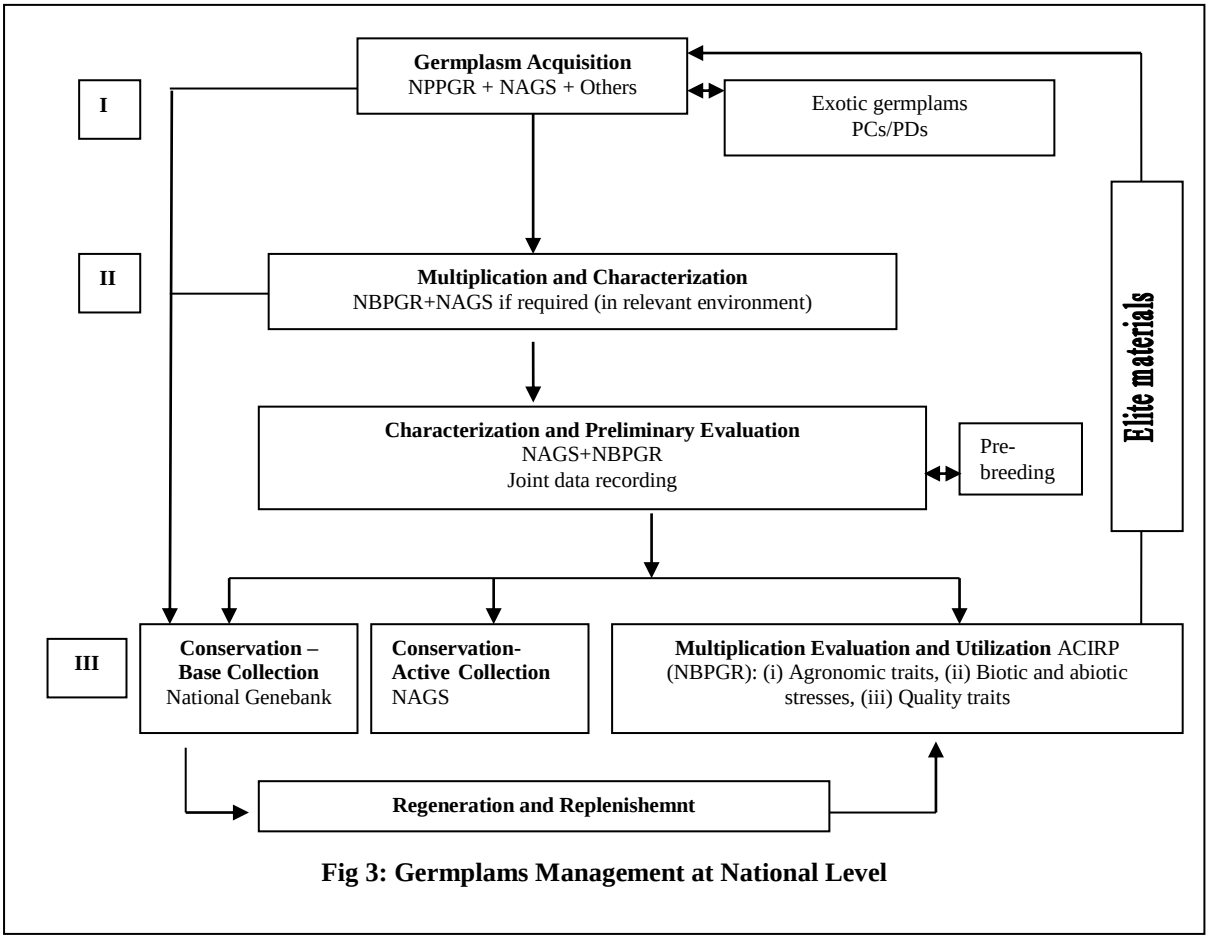



**Fig 3: Germplams Management at National Level**

Characterization and evaluation are essential to promote the utilization of materials. A large number of germplam is yet to be properly characterized and evaluated. These tasks require substantial inputs and a decentralized evaluation network.With the development in information technology, the necessary tools for collation, retrieval and dissemination of information, documentation, effective germplasm management can be done through sharing of information following a network approach.

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06 Nigeria

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10 Syria

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# INTERNATIONAL TRAINING PROGRAMME ON SEED PRODUCTION AND QUALITY EVALUATION

**(for officials of AARDO Countries sponsored by MoRD, Govt. of India)**

## Division of Seed Science and Technology, ICAR-IARI, New Delhi

**(January 14-28, 2018)**

**PROGRAM SCHEDULE**

| Day / Time | Topic of the Lecture/Practical | Resource Person |
|---|---|---|
| **Day 1: Sunday (14.01.2018)** | | |
| Arrival in India and Lodging in Hotel | | |
| **Day 2: Monday (15.01.2018)** | | |
| 09:00 to 09:45 | Registration and Inauguration | |
| 09:45 to 10:00 | **Coffee/Tea Break** | |
| 10:00 to 11:00 | Seed Industry: Present status and future prospects | *Dr. K .V. Prabhu*<br>*Joint Director (Research)* |
| 11:00 to 12:00 | Variety development, evaluation and release procedure in India | *Dr. A.K. Singh*<br>*Head, Division of Genetics* |
| 12:00 to 13:00 | Regulatory framework for seed quality assurance in India | *Dr. D.K. Yadava*<br>*Head, DSST* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 15:00 | DUS testing: principles and procedures | *Dr. R.K. Choudhury*<br>*Former OSD, DSR, Mau* |
| 15:00 to 15:15 | **Coffee/Tea Break** | |
| 15:15 to 17:00 | Principles and procedures of seed sampling Practical Session: Sampling using Mechanical divider, Boerner divider, Gamet divider, Soil divider, Random cup method, Hand halving method, Spoon method) | *Dr. K. K. Singh*<br>*Senior Scientist &*<br>*Mr. Yogendra Singh, CTO, DSST* |
| **Day 3: Tuesday (16.01.2018)** | | |
| 09:30 to 10:30 | ISTA Accreditation – Procedure and benefits | *Dr. S. K. Lal*<br>*Principal Scientist, DSST* |
| 10:30 to 11:30 | Principles and practices of seed production in self pollinated crops | *Dr. Shiv K. Yadav*<br>*Principal Scientist, DSST* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Principles and practices of seed production in cross pollinated crops | *Dr. S.K. Chakrabarty*<br>*Principal Scientist, DSST* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 17:00 | Visit to IARI Museum and National Agricultural Science Museum, NASC Complex | *Mr. Pawan Kumar Khera*<br>*Technical Officer, DSST* |
| **Day 4: Wednesday (17.01.2018)** | | |

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|---|---|---|
| 09:30 to 10:30 | Principles and practices of seed production in wheat and barley | *Dr. Sanjay Kumar*<br>*Principal Scientist &*<br>*In-charge, SPU* |
| 10:30 to 11:30 | Principles and practices of seed production in Maize | *Dr. Firoz Hussain*<br>*Senior Scientist,*<br>*Division of Genetics* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Visit to Seed Production Unit fields | *Dr. Gyanendra Singh*<br>*Principal Scientist, SPU* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 17:00 | Seed production of vegetable and ornamental crops under protected cultivation including field visit | *Dr. P.K. Singh*<br>*Principal Scientist, CPCT* |
| **Day 5: Thursday (18.01.2018)** | | |
| 09:30 to 10:30 | Principles and practices of seed production in soybean | *Dr. Monika A. Joshi*<br>*Principal Scientist, DSST* |
| 10:30 to 11:30 | Principles and procedures of physical purity analysis | *Dr. S.K. Jain*<br>*Professor, DSST* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Practical Session on Determination of different purity components, use of purity tolerance tables, calculation & reporting of results | *Dr. S.K. Jain*<br>*Professor &*<br>*Mr. Anuj Kumar, TA, DSST* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 18:00 | Introduction to Genetic Purity Testing: Importance, Principles, Tools and Essential Protocols and Practical Session on varietal and genetic purity testing and GM Seed Testing (DNA extraction; Quantification; amplification; horizontal electrophoresis, Gel documentation and other GM seed testing methods | *Dr. Arun Kumar M.B.*<br>*Principal Scientist &*<br>*Dr. Ravish Kumar, TA, DSST* |
| **Day 6: Friday (19.01.2018)** | | |
| 09:30 to 10:30 | Principles and practices of seed production in Sugarcane | *Dr. Bakshi Ram*<br>*Director, SBI, Coimbatore* |
| 10:30 to 11:30 | Public-Private partnership in seed sector: An Indian Experience | *Dr. Aravind Kapur,*<br>*MD, Acsen HyVeg Pvt. Ltd.* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Participatory seed production: a successful model for dissemination of varietal technology | *Dr. S. A. Patil*<br>*Former Director, IARI* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 17:00 | Principles & procedures of germination and seed moisture testing and Practical Session on Different germination | *Ms. Usha Rani Pedireddi*<br>*Scientist, DSST &* |

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|---|---|---|
| | methods; Moisture testing and reporting of results | *Mrs. Manisha Saini, TA, DSST* |
| **Day 7: Saturday (20.01.2018)** | | |
| 09:30 to 11:30 | Principles and practices of seed production in Brassicaceae species including field visit | *Dr. D.K. Yadava Head, DSST & Dr. Naveen Singh, Principal Scientist, Division of Genetics* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Principles and practices of seed production in Cotton | *Dr. Navinder Saini Senior Scientist, Division of Genetics* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 16:00 | Visit to National Phytotron Facility | *Mr. Nanda Kumar C.T.O., NPF, IARI* |
| 16:00 to 17:00 | Visit to National Phenomics Facility | *Dr. C. Viswanathan, Head, Division of Plant Physiology* |
| **Day 8: Sunday (21.01.2018)** | | |
| 06:00 to 20:00 | Visit to Seed Certification Agency, Agra | *Mr. M. Athar TA, DSST* |
| **Day 9: Monday (22.01.2018)** | | |
| 09:30 to 10:30 | Principles and practices of seed production in Sorghum and millets | *Dr. S.P. Singh Principal Scientist, Division of Genetics* |
| 10:30 to 11:30 | Principles and practices of seed production in vegetable crops | *Dr. B. S. Tomar Head, Division of Vegetable Science* |
| 11:30 to 11.45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Visit to Vegetable seed production plots | *Dr. Zakir Hussain, Principal Scientist, Division of Vegetable Science* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 17.00 | Seed Vigour and viability testing: Concept, Principles and Methods and Practical Session on Seed Vigour and Viability Testing (TZ test: Staining, different Viability Groups in different Crop Species; EC Test, AA Test: Procedure & Precautions) | *Dr. Sudipta Basu Principal Scientist, & Dr. Veena Vashist, CTO, DSST* |
| **Day 10: Tuesday (23.01.2018)** | | |
| 09:30 to 10:30 | Role of International organizations in seed production and quality evaluation | *Dr. P.K. Agrawal* |

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|---|---|---|
| | | *MD, Prasha Agri-Consultants Pvt. Ltd, New Delhi* |
| 10:30 to 11:30 | Plant variety protection: Indian Prospective | *Dr. R. C. Agrawal*<br>*Registrar General, PPVFRA* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Principles and practices of seed production in forage crops | *Dr. D. Vijay*<br>*Senior Scientist, DSST* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 17:00 | Exposure visit to Gene Bank at NBPGR | *Dr. Chitra Pandey*<br>*Principal Scientist, NBPGR* |
| **Day 11: Wednesday (24.01.2018)** | | |
| 09:30 to 10:30 | IARI Extension activities with special reference to seed production | *Dr. J. P. Sharma*<br>*Joint Director (Extension)* |
| 10:30 to 11:30 | Seed quality assurance system: A Global perspective | *Dr. Jai Singh*<br>*MD & CEO*<br>*Sakata Seed India Pvt. Ltd.* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Seed health testing: molecular approaches | *Dr. S. Nagamani*<br>*Scientist, DSST* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 17:00 | Seed quality assurance: SPS and quarantine Issues and Practical Session on detection of seed-borne diseases | *Dr. Atul Kumar*<br>*Principal Scientist &*<br>*Sh. Dharam Pal, TA, DSST* |
| **Day 12: Thursday (25.01.2018)** | | |
| 09:30 to 11:30 | Principles and practices of seed production in pulse crops and field visit | *Dr. S.K. Lal*<br>*Principal Scientist, DSST* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Seed quality enhancement – Principles & procedures | *Dr. Sushil Pandey,*<br>*Principal Scientist, NBPGR* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 17:00 | Visit to National Seeds Corporation and interaction with CMD NSC. | *Mr. Kuldeep Singh, Senior General Manager, NSC* |
| **Day 13: Friday (26.01.2018)** | | |
| **Visit to ICAR-IARI, Regional Station Karnal** | | |
| 09:00 to 17:00 | Varietal Maintenance and practical aspects in seed production of vegetable Crops | *Dr. V.K. Pandita*<br>*Head, IARI-RS, Karnal &*<br>*Technical staff* |
| | Maintenance of Varieties in rice and rabi pulses | *Dr. Rakesh Seth*<br>*Principal Scientist* |
| | Maintenance of varieties in wheat and kharif Pulses | *Dr. R.N. Yadav*<br>*Principal Scientist* |

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|---|---|---|
| | Post-harvest handling, processing and safe storage of seed | *Dr. Ashwani Kumar*<br>*Senior Scientist* |
| | Good agricultural practices for quality seed production | *Dr. N. K. Chopra*<br>*Principal Scientist* |
| **Day14: Saturday (27.01.2018)** | | |
| 09:30 to 10:30 | Seed deterioration: physiological aspects | *Dr. Tej Pal Singh,*<br>*Senior Scientist, DSST* |
| 10:30 to 11:30 | Importance and use of tolerances in seed testing | *Dr. Shiv K. Yadav*<br>*Principal Scientist, DSST* |
| 11:30 to 11:45 | **Coffee/Tea Break** | |
| 11:45 to 13:00 | Seed production system in India | *Dr. Dinesh K. Aggarwal,*<br>*Director, IISS, Mau (UP)* |
| 13:00 to 14:00 | **Lunch Break** | |
| 14:00 to 16:00 | Feedback and wrap programme | |
| 16:00 to 16:45 | Valedictory Function | |
| 16:45 to 17:00 | **Hi-Tea** | |
| **Day 15: Sunday (28.01.2018)** | | |
| Departure of trainees | | |

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