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National Training
on
QUALITY SEED PRODUCTION OF VEGETABLES
(November 14-18, 2022)

Training Manual

Organized by:

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

National Seed Research and Training Centre
Collectry Farm, G. T. Road Varanasi-221 106 (U.P.)
Phone: 0542-2370222, Fax: 0542-2370298
Website: www.nsrtc.nic.in

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Course Director

(Dr. M. P. Yadav)
Coordinator to Director

Training Coordinators

Mr. Anil Varma Nalla
Junior Seed Analyst

Er. M K Viswakarma
Seed Processing Engineer

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

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Compiled & Edited by:

Er. M. K. Vishwakarma, Seed Processing Engineer

Mr. Anil Varma Nalla, Junior Seed Analyst

Dr. A. K. Verma, Senior Seed Analyst

Mrs. Ekta Kumari, Senior Seed Analyst

Mr. Javesh Kumar, Junior Seed Analyst

NATIONAL SEED RESEARCH AND TRAINING CENTRE
VARANASI-221 106 (UTTAR PRADESH)

FOREWORD

Vegetables are important food and extremely essential for the maintenance of health and prevention of diseases, therefore, valued for their high carbohydrate, vitamin, mineral and fiber contents. India is a leading vegetable producing country in the world occupying around 27.48 million hectare area under vegetable cultivation with the annual production of 334.60 million tonnes. India is blessed with the unique gift of nature of diverse climate and distinct seasons, make it possible to grow an array of vegetables number exceeding more than hundred types.

In the recent past, Indian agriculture witnessed tremendous progress in vegetable production. One of the major factors which influence this achievement in vegetable crops is the Quality Seed. The most important approach to enhance the vegetable crop productivity is production of quality seeds and making them available to the common farmers in sufficient quantity. The importance of good quality seed can hardly be over emphasized as it is crucial for higher productivity.

National Seed Research and Training Centre, Varanasi is organizing a National Training Programme on "Quality Seed Production of Vegetable Crops" during November 14-18, 2022. The prime objective of this training is to update the knowledge of all participants who are engaged in vegetable seed production, quality control and to provide a forum to discuss and exchange their knowledge to enhance the production and availability of quality seeds of vegetables amongst farming community across the country.

This training module consists of valuable information on various aspects of seed to seed system in vegetables. I hope this compilation will serve as a useful resource book and guide to all concerned.

Date: 18 .11.2022

Place: Varanasi

(Dr. M. P. Yadav)
Coordinator to Director

National Training

on

QUALITY SEED PRODUCTION OF VEGETABLES

(NOVEMBER 14-18, 2022)

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NSRTC at a glance……

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

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

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

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

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

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

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

PRIME OBJECTIVES:

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

VISION:

Our vision is to

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

MISSION:

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

STRATEGY:

NSRTC pursues its Mission and Goals through:

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

Staff strength:

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

S.No.	Name of Post	Staff strength
1.	Director	1
2.	Chief Seed Analyst	1
3.	Seed Processing Engineer	1
4.	Seed Technologist	3
5.	Sr. Seed Analyst	2
6.	Administrative/Accounts Officer	1
7.	Jr. Seed Analyst	5
8.	Private Secretary	1
9.	Stenographer	2
10.	Librarian	1
11.	Assistant (Administration/ Accounts)	1
12.	Caretaker cum Storekeeper	1
13.	Lower Division Clerk	1
14.	Laboratory Attendant	2
Total		**23**

NSRTC is especially designed for continuous dissemination of knowledge of seed and thereby improve skill, competency and scientific soundness of individuals engaged in seed development programme. NSRTC regularly organizes training on various aspects of seed for the officials working in Seed Certification Agencies (25 in number), Seed Testing Laboratory (147 in number), Seed Law Enforcement Agencies, Agricultural Universities and other institutes

dealing with seeds. The NSRTC, Central Seed Testing Laboratory acts as a referral lab under clause 4(1) of the Seeds Act, 1966. CSTL, NSRTC is testing more than 20,000 samples per year and performs at par with ISTA (International Seed Testing Association) with regard to seed testing net work in the country.

National Seed Testing Laboratory as Central Seed Testing Laboratory

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

Grow Out Test

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

NSRTC is geared to go Global

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

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

Dr. M. P. Yadav
Coordinator to Director

Detection of seed borne pathogens

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

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

Conventional seed detection assays

Testing seeds for plant pathogen scan be a difficult task. Unlike infected vegetative plant tissues, infested seed scan be asymptomatic, making visual detection impossible. Additionally, pathogen populations on seeds may below, and infested seeds may be non-uniformly distributed within a lot. Many detection assays exist for different seed borne pathogens, however, few satisfy the minimum requirements for adequate seed tests. Ideally, seed assays should be sensitive, specific, rapid, robust, inexpensive and simple to implement and interpret. Seed assays have been developed based on different technologies including visual examination; selective media; seedling grow-out tests and serological techniques. While these tests have been used for many years, some of them have shortcomings that make them less than ideal. Brief descriptions of these assays including their advantages and disadvantages are discussed below.

Visual examination

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

Selective media

A direct method of testing seeds is by allowing pathogens to grow from them onto appropriate artificial media. This can be done by directly plating surface-sterilized seed samples or seed-wash liquid onto artificial media, followed by incubation under adequate conditions. Once a pathogen is isolated it can be identified by its cultural or bio chemical characteristics e.g. the production of a bluish-green fluorescent pigment on King's B medium in the case of fluorescent *Pseudomonas* spp. or the production of dark, muriform conidia in the case of *Alternaria* spp. Unfortunately, seeds may be contaminated by saprophytic microorganisms (non pathogens)

that grow as well as, or better than target organisms on nutrient-rich, artificial media. The excessive growth of saprophytic organisms including *Rhizopus* spp., *Penicillium* spp., and yeasts make it impossible to identify pathogens that may be present. The inability to identify the unique characteristics of the target pathogens in the presence of contaminating microorganisms lead to inaccurate assessments of seed lot infestation. To overcome this problem, selective artificial media are developed that use antibiotics, fungicides, selected carbon and nitrogen sources and other inhibitory compounds to retard the growth of non target micro flora while allowing the pathogen to grow. Many selective and semi selective media have been developed for seed borne fungi and bacteria. Unfortunately, development of such media is time consuming and requires specific knowledge of the nutritional requirements and chemical tolerances of the target organism, relative to the non-target seed micro flora. Employing selective media also requires 2 to 4 d for pathogen growth and the test operator must be familiar with the range of cultural characteristics associated with the pathogen. Finally, while selective media can be applied for certain bacteria and fungi, it cannot be applied for non culturable obligate parasites, e.g., viruses, nematodes and certain fungi and bacteria.

Seedling grow-out assay

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

Blotter method

The collected seed samples need to analyze for the presence of major seed borne fungal the pathogens by blotter method following the International rules for Seed Testing. Seeds are tested for each variety maintaining four replications. Twenty-five seeds are placed on three layers of moist blotting paper (Whatman No.1) in each glass petridish. The petridishes are incubated at 25±1°C under 12/12 hrs light and darkness cycle for 7 days. Each seed is observed under strereo microscope in order to record the presence of fungal colony and bacterial ooze 7 days after incubation based on growth habit. In doubtful cases temporary slides are prepared from the

fungal colony observed under compound microscope. Appropriate keys are consulted for identification of the fungi and bacteria. The results are presented as percent incidence for individual pathogen. Germination of the seeds is also recorded. Each individual incubated seed is observed under stereomicroscope in order to record the incidence of seed borne fungi. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper. For proper identification of fungi temporary slides are prepared from the fungal colony and observed under compound microscope and identified with the help of Keys. The fungi from the incubated seeds are also transferred to PDA when needed. The culture is incubated at 25±10°C for 3-7 days. Temporary semi permanent slides are prepared from the fungal colony and observed under compound microscope. The fungi are identified with the help of different books, manuals and publications. The results are presented as percent incidence for individual pathogen. acronyms written on the reverse.

Rolled paper towel method

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

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

Agar plate method

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

Serology-based assays

Serological seed assays rely on antibodies (polyclonal or monoclonal) generated against unique antigens on the surfaces of plant pathogens. Antibodies bind strongly and specifically to their

antigens and can subsequently be detected by the enzymatic digestion of substrates or fluorescent tags. Serology-based seed tests have several formats including the widely applied enzyme-linked immunosorbent assay (ELISA) and immune fluorescence microscopy. Serological assays do not require pure isolations of the pathogen and, hence, are applicable to biotrophic and necrotrophic seed borne pathogens. Currently serology is the most widely used detection assay for seed borne viruses and it has proven to be sensitive and robust. Serology has also been widely used for the detection of bacterial and fungal plant pathogens, but the unavailability of species-specific antibodies is a limitation. Additionally, the detection thresholds of serology-based assays vary significantly based on the quality of the antibody and the testing format. Finally, with serology-based assays it is possible to detect nonviable pathogens which results in erroneous (false-positive) interpretation.

PCR-based assay

PCR-based assays exhibit very higher levels of sensitivity than any other conventional techniques. They require extraction of PCR-quality DNA from the target organisms in the background of saprophytic organisms and inhibitory seed-derived compounds when applied to seed tests.PCR consists *in vitro* enzymatic amplification of an initial quantity of target DNA from any living organisms including fungi, bacteria and viruses. However, due to its specificity, speed and sensitivity it has been used to diagnose many seed borne pathogens. However, high capital costs and technical expertise for establishing PCR capabilities is major obstacle in the PCR-based detection technique. The second major obstacle in successful implication of this method is false negatives (inhibition of PCR reaction by various compounds contained in seeds) and false positives (amplification of DNA from non-viable cells) which restricts the accurate detection of the pathogen. Along with this its incapability in distinguishing between viable and non-viable cells is also one of the major constraints of this method.

Some Important Bacterial Seed Borne Pathogens

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

Some Important Fungal Seed Borne Pathogens

Crops	Diseases	Pathogens
Wheat	Loost smut	*Ustilago segetum* var. *tritci*
	Karnal smut	*Neovossia indica*
	Flag smut	*Urocystisagropyri*
Chickpea	Ascochyta blight	*Ascochyta rabiei*
	Wilt	*Fusarium oxysporum*f.sp.*ciceri*
Crucifers	Grey and black leaf	*Alterniabrassicae*
	Spot	*A. brassicicola*
Rice	Bunt	*Neovossia horrid*
	False Smut	*Ustilaginoidea virens*
	Stackburn	*Pyriculariaoryzae*
		Trichoconiellapadwickii
Cotton	Anthracnose	*Colletotrichum indicum*
	Wilt	*F.oxysporumf*.sp.*vasinfectum*
Maize	Black kernel rot	*Botryodiplodiatheobromae*
	Cob rot	*Fusarium moniliformae*
	Southern leaf blight	*Drechlera maydis*
Pearl millet	Downy mildew	*Sclerosporagraminicola*
	Smut	*Tolyposporiumpenicillsriae*
Sorghum	Anthracnose	*Colletotrichum graminicola*
	Kernel or grain smut	*Sphacelothecasorghi*
	Downy mildew	*Peronosclerosporasorghi*
Soybean	Anthracnose	*Colletotrichum dematium*
	Pod & stem blight	*Phomopsis sojae*
	Purple seed stain	*Cercosporakikuchii*
Cucumis spp.	Anthracnose	*Colletotrichum lagenarium*
Brinjal	Fruit rot	*Phomopsis vexans*
Onion	Damping off	*Botrytis allii*
	Downy mildew	*Peronospora destructor*
	Purple blotch	*Alterniaporri*
	Stemphylium Blight	*Stemphylium vesicarium*
Pepper chilies	Anthracnose	*Colletrotrichumcapsici*
	Or ripe fruit rot	

Tomato	Buck eye rot	*Phytophthora parasitica*
	Damping off	*Phythiumaphanidermatum*
	Early Blight	*Alterniasolani*
	Late blight or Fruit rot	*Phytopthorainfestans*

Seed treatment and seed biopriming: Principles and Procedures

B.K. Sarma

Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi

Agriculture relies on the growth of seeds. They are the first stage in the lifecycle of a crop and if seeds fail to germinate, crops fail. Seeds face a lot of challenges from diseases to pests to environmental stresses. While seeds can overcome these on their own, the chances of success can be improved with seed enhancement. Various modes of application of beneficial microbes onto plants are described. Among those, seed treatment, seed biopriming, soil treatment, seedling root dip treatment and foliar application are the most common methods. Seed treatment is considered as one of the most effective ways to support the growth of seeds and reduce the challenges that they face. Seed treatment refers to the application of biological, physical and chemical agents and techniques that can be applied to seeds to provide them protection from seed-borne pathogenic organisms and storage insects. Seed treatment can also encourage healthy crops by improving their immunity and promoting uniform germination. While traditional crop protection methods that are applied on a broader level to the crops have their place, with seed treatment, the needs of every individual seed can be met. Seed treatment enhances the resistance of the seeds, making them stronger against pest attacks and stresses in their environment. Chemical or biological seed treatments can provide critical protection since the germination stage, protecting the emergence out of the soil and during the first stage of the crop cycle by preventing seeds against soil-borne pathogens, seed-borne insects, diseases, and pests.

Benefits of Seed Treatment:

There are many benefits of seed treatment that are making them popular with farmers. Some of these benefits are as follows:

- Seed treatment protects seeds from insects & diseases
  One of the foremost benefits of seed treatment is the additional protection that it provides to the seeds. During the early stages of growth, it is critical that seeds don't fall prey to diseases or pests or else they will fail to germinate. With untreated seeds, there is also a chance of plant diseases spreading which is something that can be prevented when they are treated to improve their immunity.
- Seed treatment controls soil insects
  Seed treatment is not only beneficial for seeds but also for the soil. It can control soil insects and also add much-needed nutrients and valuable microorganisms that help the plants grow.
- Seed treatment improves germination
  Germination of a seed depends on a variety of factors but the chances of germination can be improved with seed treatment. Since seed treatment is equally applied to all the seeds, it can also promote uniform germination of the crop. Seed Treatments formulations are special and safe for crops and were designed in order to avoid any

potential damage such as delay in germination or irregular emergence, neither phytotoxic effect than un appropiated formulations for other uses in the field may cause.

- Early and uniform establishment and growth
  Seed treatment ensures early germination of the seeds and the crop establishment is generally uniform. It is possible due to care been taken by the chemicals used for seed treatment to protect them from the harmful pests and pathogens.
- Enhances nodulation in legume crop
  Healthy seed promotes higher nodulation as well. It is because the nodulating bacteria does not have to encounter the insect pests of pathogens to nodulate. Hence, it is normally observed that seed protection leads to higher nodulation in leguminous crops.
- Provides protection from storage insects
  Storage insects is a big issue for commercial seed production. As the harvested seeds has to be stored till the next cropping season it is important that seeds are protected in storage from stored grain pests and remain viable for germination. Seed treatment helps is achieving the goal.
- Controls soil insects
  The chemicals used for seed treatment are also important for managing soil insects. Therefore, dual objectives could be achieved through seed treatment by managing stored grain pests as well as soil insects that can be potential pests.

- Microbial seed treatment
  One of the latest developments is microbial seed treatments. Microbial seed treatments can reduce fertilizer and pesticide applications and increase yields. Different beneficial fungi and bacteria are used to treat seeds in order to obtain the desired benefits. Microbial seed treatments have the advantage that less microbial material is needed, thus reduces the cost. Microbial inoculants were developed by institutions and companies and the seeds treated with microbes have enriched carbon starved soils, provided drought resistance, decreased fertilizer and pesticide applications, and increased crop yields.

Types of Seed Treatment:

Modern seed treatments and seed processing has come a long way in the past few decades. More and more farmers are using commercial seed treatments in agriculture across the world. For some, pre-treated seeds are also an option that can be directly used without the need to apply the treatment yourself. The importance of seed treatment and its many benefits cannot be overlooked as they give farmers more opportunities to reduce the risk of failure for their crops. There are many types of commercial seed treatments available today. Depending on the need of the specific crops, farmers can pick a single or a combination of multiple seed treatments with chemicals or biological agents. Seeds are especially vulnerable to fungi in the early stages of growth and some fungi can be hard to combat on their own. Fungicide seed treatment can provide them protection and prevent fungal diseases. Pests are another concern that farmers have to factor in while looking at the early stages of growth. Many insects target seeds and can damage them before they even germinate. Insecticide seed treatments can provide protection against such pests with the added benefit of having a lower concentration of chemicals than the pesticides applied to fully-grown crops. Microbial inoculants are seed treatment products that can stimulate plant growth, promote soil biodiversity, and even address specific issues such as nitrogen fixation in legumes. With this beneficial microorganisms are delivered directly to the area where the plant interacts with the soil, which encourages growth. With plant growth

regulators, seeds have additional assistance for germination and an enhanced tolerance for stresses during the critical early stages of growth. This seed treatment for germination is also helpful when seeds have to germinate in tough conditions. Fertilizer seed treatments are another type of seed treatment that helps with plant growth. Seed treatments with biofertilizers can enhance fertilizer performance or supply micronutrients to the soil to enrich the growth environment for the seeds.

- **Seed disinfection:** Seed disinfection refers to the eradication of fungal spores that have become established within the seed coat, or i more deep-seated tissues. For effective control, the fungicidal treatment must actually penetrate the seed in order to kill the fungus that is present.
- **Seed disinfestation:** Seed disinfestation refers to the destruction of surface-borne organisms that have contaminated the seed surface but not infected the seed surface. Chemical dips, soaks, fungicides applied as dust, slurry or liquid have been found successful.
- **Seed Protection:** The purpose of seed protection is to protect the seed and young seedling from organisms in the soil which might otherwise cause decay of the seed before germination.

Conditions under which seed must be treated

- **Injured Seeds:** Any break in the seed coat of a seed affords an excellent opportunity for fungi to enter the seed and either kill it, or awaken the seedling that will be produced from it. Seeds suffer mechanical injury during combining and threshing operations, or from being dropped from excessive heights. They may also be injured by weather or improper storage.
- **Diseased seed:** Seed may be infected by disease organisms even at the time of harvest, or may become infected during processing, if processed on contaminated machinery or if stored in contaminated containers or warehouses.
- **Undesirable soil conditions:** Seeds are sometimes planted under unfavourable soil conditions such as cold and damp soils, or extremely dry soils. Such unfavourable soil conditions may be favourable to the growth and development of certain fungi spores enabling them to attack and damage the seeds.
- **Disease-free seed:** Seeds are invariably infected, by disease organisms ranging from no economic consequence to severe economic consequences. Seed treatment provides a good insurance against diseases, soil-borne organisms and thus affords protection to weak seeds enabling them to germinate and produce seedlings.

Procedure for Seed Treatment:

Seed treatment is a term that describes both products and processes. Seed treatment can be done in one of the following types.

- **Seed dressing:** This is the most common method of seed treatment. The seed is dressed with either a dry formulation or wet treated with a slurry or liquid formulation. Dressings can be applied at both farm and industries. Low cost earthen pots can be used for mixing pesticides with seed or seed can be spread on a polythene sheet and required quantity of chemical can be sprinkled on seed lot and mixed mechanically by the farmers.
- **Seed coating:** A special binder is used with a formulation to enhance adherence to the seed. Coating requires advanced treatment technology, by the industry.

- **Seed pelleting:** The most sophisticated Seed Treatment Technology, resulting in changing physical shape of a seed to enhance palatability and handling. Pelleting requires specialized application machinery and techniques and is the most expensive application.

Precautions in Seed Treatment:

Most products used in the treatment of seeds are harmful to humans, but they can also be harmful to seeds. Extreme care is required to ensure that treated seed is never used as human or animal food. To minimize this possibility, treated seed should be clearly labelled as being dangerous, if consumed. The temptation to use unsold treated seed for human or animal feed can be avoided if care is taken to treat only the quantity for which sales are assured. Care must also be taken to treat seed at the correct dosage rate; applying too much or too little material can be as damaging as never treating at all. Seed with a very high moisture content is very susceptible to injury when treated with some of the concentrated liquid products.

Seed Biopriming:

Seed biopriming is an extended version of seed treatment. It is an emerging technique to enhance seed germination under stressed conditions. Seed biopriming with potential microbes can help in increasing colonization, proliferation, and establishment of biocontrol agents on the seed surface. Subsequently, it boosts seedling vigor and induce systemic resistance to biotic and abiotic stresses (Singh et al., 2016). Seed biopriming is even considered superior to seed treatment as it is recorded to be the most efficient and productive method as it utilize bioagents judiciously for enhancing crop productivity (Rajput et al., 2019). Seed bio-priming is a process of biological seed treatment that refers to a combination of seed hydration and inoculation of the seeds with beneficial microorganisms. In seed biopriming, primarily it conditions seed with a priming agent which consequently imparts the seedlings with an enhanced defence against environmental challenges such as biotic and abiotic stresses. In principle, the seed biopriming method involve soaking of the seeds in water for 12 h (need based according to the types of seeds used). Selected formulated product of the microorganism is added to the pre-soaked seeds at the rate of 5-10 g/kg of seed and mixed well. The treated seeds are then kept in polythene bags, heaped and covered with moist jute sack to maintain high humidity and maintained for 24-48 h at approximately 25–32 °C. During this period, the bioagent adheres the seed surface to form a protective layer all around the seed coat. These bio-primed seeds can be sown in the nursery bed.

Seed biopriming is quite economic as it requires utilization of a significantly low amount of the selected bioagent for priming the seed lot. The biological aspects of disease management and the physiological aspects of seed hydration involved in biopriming allow increased colonization, proliferation and establishment of the delivered biocontrol agents on the seed surface. Consequently, biopriming enhances nutrient and water absorption and uptake, boosts seedling vigour as well as induces systemic resistance to biotic and abiotic stresses. Seed priming with fungi and vitamins have strong effects on plant defense mechanism against bacterial pathogens. The bioprimed plants ameliorate their metabolic pathways thereby augmenting accretion of organic solutes like sugars, polyhydric alcohols, proline, betaines, polyamines, quaternary ammonium compounds and secondary metabolites, such as, polyphenols, alkaloids and flavonoids. However, the process of acclimatization of microbes onto the seed coat environment may lead to a rapid decline in microbial count. In this context, addition of plant growth regulators or root exudate components as biopriming agents may play an essential role in structuring the rhizospheric microbial communities.

Suggested readings:

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Insect Pest Management in Vegetable Seed Production

Compiled by

Keerthi MC, PVR Reddy & Manjunatha L

Scientist, Division of Crop Protection, ICAR- IIHR, Bengaluru- 560089.

Keerthimanikya@gmail.com

The growing population and the availability of rich arable lands and various agro-climatic zones in India represent one of the key factors positively influencing the market. In addition, the commercialization of agriculture and the active collaboration of private and public sectors are propelling the growth of the seed industry in the country. Apart from this, leading seed breeders are increasingly introducing advanced digital technologies to help farmers overcome the negative impacts of pests, climate, and other environmental factors. Seed is the basic and most critical input for sustainable agriculture. The response of all other inputs depends on quality of seeds to a large extent. It is estimated that the direct contribution of quality seed alone to the total production is about 15 – 20% depending upon the crop and it can be further raised up to 45% with efficient management of other inputs.

The production of quality seeds disturbed by various biotic and abiotic stresses. Among which insects pests are one of the major causes of concern. Because they not only affect the seeds quantitatively but also reduces the quality of seeds. Vegetables are important component of Indian agriculture. Diverse agroclimatic conditions of the country permit growing of several vegetables round the year. India produces about 90 million tons of vegetables (excluding potato, sweet potato, tapioca and including chilli) from about 6.72 million hectares and retains second position after China. The per capita, per day requirement of vegetables is about 300 g and thus we have to produce close to 150 tons of vegetables to meet out domestic requirements. Hence production of quality seeds of paramount importance. On average, pests account for 20-40% of yield losses worldwide, costing the global economy a combined $290 billion, according to the FAO. Here in the present chapter we have compiled the major insects' pests of vegetables, their damage symptoms and management strategies.

1. SERPENTINE LEAF MINER, *Liriomyza trifolii* (Agromyzidae: Diptera)

It is the polyphagous and very widely distributed throughout India. The adult is a tiny dark fly with yellow markings, with thin, transparent wings, females have a prominent retractile ovipositor. The maggots are legless, pale yellow, body tapers anteriorly. Maggot mines into the leaf and feeds on the mesophyll of the leaves making serpentine mines. As the larva grows the diameter of the mine increases. The white long circular mines can be seen in old leaves. Young leaves have small and thin mines in them and the incidence is first noticed at nursery stage itself.

Management:

- Initiate the spray as soon as the cotyledons are noticed (very important)because, spraying the crop after leaf miner infestation results in lack of adequate control.
- When the infestation is high, spraying at best can only kill the larvae on the leaves bug may not have much impact on pupae in soil. This is the prime reason for control failure and rain festation of *L. trifoli* on many vegetables.
- Bitter gourd (*Momordica charantia*) may be used a repellent crop around most susceptible host plants (Bittergourd is least preferred).
- Use of yellow sticky traps has also been practiced to trap *L. trifoli*, especially in greenhouse.

2. **Pinworm: *Tuta absoluta* (Gelechiidae, Lepidoptera)**

The tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the global major destructive invasive pests was found to be occurring in India in the year 2014. The pest has spread from South America to several parts of Europe, entire Africa and has now spread to India. Plants are damaged by direct feeding on leaves, stems, buds, calyces, young fruit, or ripe fruit and by the invasion of secondary pathogens which enter through the wounds made by the pest. It can cause up to 90% loss of yield and fruit quality under greenhouses and field conditions. *Tuta absoluta* prefers to feed on tomato, though other solanaceous plants, including potato, have been recorded as hosts. It is known to have many generations in a year and affects tomato in all growing stages.

Damage symptoms: The larvae of *T. absoluta* mine the leaves producing large galleries and burrow into the fruit, causing a substantial loss of tomato production in protected and open filed cultivations. The larvae feed on mesophyll tissues and make irregular mine on leaf surface. Damage can reach up to 100%. This pest damage occurs throughout the entire growing cycle of tomatoes. Tuta absoluta has a very high reproduction capability. There are up to 10-12 generations in year in favorable conditions.

Management:

- Collect and destroy the pinworm affected plants and fruits
- Avoid solanaceous crops after tomato
- Use healthy seedlings for transplanting
- Keep pheromone traps @ 10 no/ ac to attract and kill the adult moths.
- Initiate the insecticide application when trap catch reach 20-30 moth/ trap/ week.

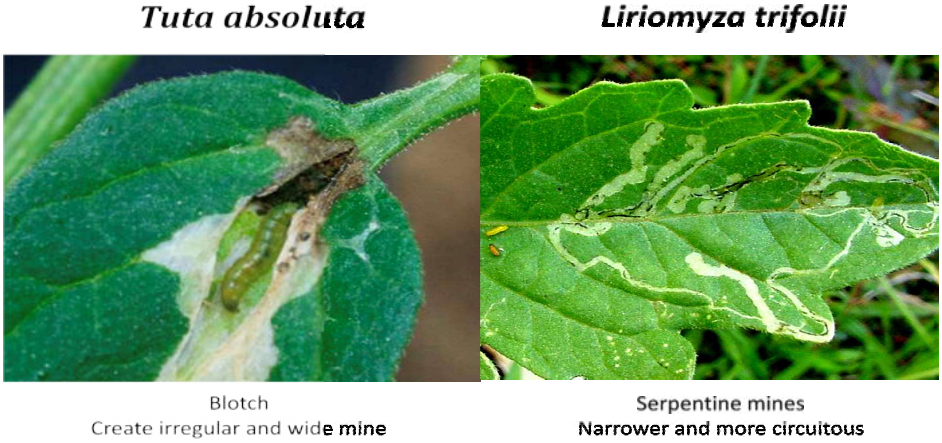

Fig 1. Difference between damage symptoms of *Tuta absoluta* and *Liriomyza trifolii*

3. **Fruit Borer: *Helicoverpa armigera*** (Lepidoptera: Noctuidae)

It is the number one pest among the insect pests of agricultural and horticulture crops in India Polyphagous and the host range is expanding.

Damage symptoms: First instars larvae initially feed on leaves before migrating to developing green fruits. The caterpillars bore into fruits by inserting only their head. Fully grown caterpillars show characteristic whitish and dark brown longitudinal stripes. Larvae more from one fruit to another and may destroy many fruits. External symptoms appear on the form of a bored hole.

Management:

- Planting 45 day old African marigold as a trap crop (*Tagetus erecta*) at the time of transplanting tomato for every 14 rows. (Male female are attracted to marigold flowers and lay eggs) to synchronies flowering, plant 40 and 25 day old seedlings of marigold and tomato, respectively at a time.
- Monitor pest through pheromones traps Helilure at 12/ha
- Place 15-20 bird perches (T shaped) per ha to invite insectivorous birds.

T. absoluta *H. armigera*

Pin hole on fruit Circular hole on fruit

Fig 2. Difference between damage symptoms of *T. absoluta* and *H. armigera*

***Tuta* control**								
sl no	**Chemical name**	**Company**	**Active ingredient**	**concentration**	**Per acre**	**Packing**	**cost**	**MOA**
1	Deligate	Dow	Spinetoram 11.7% SC	1 ml/ lit	250 ml	180 ml	2124	Systemic
2	Moratr	Dhanuka	Carap hyrdohcoride	1.5 gm/ lit	400 ml	500 gm	1250	Systemic
3	Tracer	Dow	Spinosad	0.4ml/ lit	100 ml	75 ml	1557	Systemic
4	Pendal	Coramandal	Penthoate	2 ml/ lit	500 ml	1 lit	1125	Systemic
5	Benevia	FMC	Cyantraniliprole	0.5 ml/lit	125 ml	180	1750	Systemic
6	Larvin	Bayer	Thiodicarb 75 WP	1-1.5g/ lit	400 g	500 gm	1600	Systemic
7	Lancer gold	UPL	Acephate 500 + Imidacloprid 18	2 g/ lit	500 g	500 gm	450	Systemic
8	Coragen	Dupont	Chlorantraniliprole 18.5 % w/w	0.3 gm/ lit	75 ml	60 ml	900	Systemic

4. **Tomato whitefly, *Bemisia tabaci* (Aleyrodidae: Hemiptera)**
   - Vector of yellow leaf curl virus.
   - Nymphs and adults suck the sap on the ventral surface of leaves.
   - The affected leaves wrinkle and curl downwards.

5. **Leaf hoppers, *Amrasca biguttula biguttula* (Ishida) (Cicadellidae: Hemiptera)**

- Eggs are inserted in the leaf tissues on the upper leaf surface.
- Nymphs are also pale greenish in colour like the adults but are wingless and are found in large numbers on lower surface of leaves.

Nature & symptoms of damage:

- Both nymphs and adults inflect the same type of damage.
- They suck up the cell sap from the plant tissue.

Management:

- Seed treatment with imidacloprid 70WS (Confidor) @ 5g/kg seeed or thiamethaxam 25WG (Actara) @ 5g/kg seed for all early sucking pests.
- Keep yellow pan / sticky traps @ 25 traps/ha, for both monitoring and also mass trapping.
- Locally available empty yellow palmoline tins coated with grease / vaseline / castor oil on outer surface may also be used.

Sl no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Thrips, Aphid, whitefly								
1	Actara	Syngenta	Thiamethoxam 25 % WG	0.5g	100 g	100g	400	Systemic
2	Deligate	Dow	Spinetoram 11.7% SC	1 ml	250 ml	180 ml	2124	Systemic
3	Tracer	Dow	Spinosad 45SC	0.4ml	100 ml	75 ml	1557	Systemic
4	Confidor	Bayer	Imidacloprid 200 SL (17.8 % w/w)	0.75 ml	187 ml	250ml	820	Systemic
5	Confidor super	Bayer	Imidacloprid 30.5 SC	0.3-0.4	100 ml	100 ml	595	Systemic
Combination of pests (Leaf miner, Aphids, Thrips, Whitefly, Fruit borer)								
6	Benevia	FMC	Cyantraniliprole 10.26 % OD	0.5 ml/lit	125 ml	180	1750	Systemic
7	VOLIAM FLEXI	Syngenta	Chlorantraniliprole 08.80 % + Thiamethoxam 17.50 % w/w SC	0.5 ml/lit	80 ml	100	822	Systemic (BS)

Combination of Insect and Mite								
8	Dammu	Indofil	Propargite 50 % + Bifenthrin 5 % w/w SE	2ml/ ltr	400 ml	250 ml	565	Contact

Pests of brinjal:

1. **Brinjal shoot & fruit borer, *Leucinodes orbonalis* (Pyralidae: Lepidoptera)**

From the point of view of extent and severity of damage, various entomologists have assigned first place to *Leucinodes orbonalis*.

Damage symptoms:

- In the early stages of the growth of the plant, the larvae of shoot and fruit borer attack and kill growing shoots.
- During the fruiting season, they bore inside the fruit and by their feeding and due to presence of fecal matter, the effected fruits unfit for market.
- It is also reported that in the borer infested fruits, the reduction in vitamin C content (Ascorbic acid) goes up to 68 per cent. The pest infestation may go as high as 70% in brinjal.
- The caterpillar bores into petioles, midribs of large leaves and the shoots in early stage eat the internal tissues. When the infestation is on terminal shoots, they bend down and wither and affected shoots wither and droop.

Management:

- Remove and destroy the affected tender shoots, fallen fruits and fruits with bore holes
- Avoid continuous cropping of brinjal crop (avoid monocropping)
- Install pheromone traps (Lucin lure) @12/ha
- Use light traps @ 1/ha, to attract adults.

Sl no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Thrips, Aphid, whitefly								
1	Coragen	Dupont	Chlorantraniliprole 18.5 % SC	0.3 ml/ lit	75 ml	60 ml	900	Systemic
2	Proclaim	Syngenta	Emamectin benzoate 05 % SG	0.4g/ ltr	100g	100g	650	Contact
3	Takumi	Tata Rallis	Flubendiamide 39.35 % w/w SC	0.6g/ ltr	150g	100	699	Contact (T)
4	Tracer	Dow	Spinosad 45SC	0.4ml/ lit	100 ml	75 ml	1557	Systemic

2. **Ash weevils: (Curculionidae: Coleoptera)**

Myllocerus subfasciatus - Green in colour
M. discolor - Brown with white spots

M. viridanus - Small Light green
M. maculosus - Greenish white with dark lines on elytra

Symptoms of damage:

- Adults feed on foliage, causing ladder like cuttings along the leaf margin.
- Later adults lay eggs close to ground and the emerging grubs feed on tender roots.
- Affected plant wilts and dries up.
- Damage is noticed initially on the young seedlings and at flowering stage also.

Management

- Collect and destroy adult weevil.
- In endemic area, apply Neem cake @ 500 kg/ha at the time of last ploughing
- Application of Chlorantraniliprole 0.4% GR (Ferterra) at 4 kg / acre at the base of the plants

3. Sucking pests

3a. **Leafhopper:**
Both nymphs and adults inflect the same type of damage.
They suck up the cell sap from the plant tissue.
Vector of little leaf of brinjal (Amrasca devastans)

3b. **Whitefly:** ***Bemisia tabaci***
Nymphs and adults suck the sap on the ventral surface of leaves. The affected leaves wrinkle and curl downwards.

3c. **Mites:**
Leaves put forth a characteristic blotching appearance; become whitish then brown patches appear.

3d**. Thrips**
Characteristic silvery patch.

Sl no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Fruit & Shoot borer, Thrips & Jassids								
1	Exponus	BASF	Broflanilide 20% SC	0.1 ml/ ltr	25 ml	34 ml	2244	Contact (T)
2	Sefina	BASF	Pyriproxyfen 05 % + Fenpropathrin 15 % EC	2ml/ ltr	500 ml	400 ml	1288	Systemic
Whitefly, Shoot and Fruit borer, Mites								
3	Meothrin	Sumimato	Fenpropathrin 30 % EC	0.5ml/ ltr	125 ml	250 ml	399	Contact (T)
Whitefly & Jassids								
4	Movento	Bayer	Spirotetramat 15.31 % w/w OD	2ml/ ltr	500 ml	500ml	2024	Contact (T)
Whitefly								

5	Actara	Syngenta	Thiamethoxam 25 % WG	0.5g/ ltr	125g	250g	776	Systemic

INSECT PESTS OF OKRA

1. Bhendi shoot & fruit borer, *Earias vittella, Earias insulana* (Noctuidae: Lepidoptera)

- Widely distributed throughout India also found infesting fruit and a large number of wild and cultivated malvaceous plants.
- Summer crop suffers more losses.
- It is estimated about 69% loss in marketable yield due to attack of this insect on okra

Nature & symptoms of damage*:*

• When the crop is young, *larva bores into terminal shoots* of young plants leading death of the shoots and subsequent development of side shoots and branches.

• Larva also bores into the flower buds flowers. Infected parts will shed. Entrance hole is plugged with excreta.

• In fruits, the larvae bore inside fruits and feed on inner tissues which become deformed in shape with no market value.

• Young fruits draw flaring up symptoms and drop.

Management:

- Deep ploughing.
- Clean cultivation (timely crop termination, destruction of crop residues and bhendi stalks)
- Pre-planting cleanup measures during off-season (cleaning bunds, to remove alternate hosts etc.)
- Avoid cotton fields for bhendi cultivation, because the pests which attack cotton will also attack bhendi.
- Avoid mono-cropping of bhendi year after year.
- Collection and destruction of effected plant parts. The infested fruits and shoots should be removed regularly and buried deep in the soil.
- Use light traps @ 1/ha, to attract adults.
- Pheromone traps @ 12/ha attracts males (change - 40D).

2. Sucking pests: Whitefly; Thrips; Jassids

2a. Leafhopper:

Both nymphs and adults inflect the same type of damage.

They suck up the cell sap from the plant tissue.

Vector of little leaf of brinjal (*Amrasca devastans*)

2b. Whitefly: Bemisia tabaci

Vector of yellow mosaic virus

Nymphs and adults suck the sap on the ventral surface of leaves.

The affected leaves wrinkle and curl downwards.

2c. Thrips

Characteristic silvery patch.

Sl no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Shoot & Fruit borer								
1	Coragen	Dupont	Chlorantraniliprole 18.5 % SC	0.3 ml/ lit	75 ml	60 ml	900	Systemic
2	Proclaim	Syngenta	Emamectin benzoate 05 % SG	0.4g/ ltr	100g	100g	650	Contact (T)
Fruit borer (*Helicoverpa armigera*), Thrips (*Thrips tabaci*) and Jassids (*Amrasca devastans*)								
3	Exponus	BASF	Broflanilide 20% SC	0.1 ml/ ltr	25 ml	34 ml	2244	Contact (T)
4	Gracia	Godrej	Fluxametamide 10% EC	0.8 ml/ ltr	160ml	100 ml	1250	Contact (T)
Whiteflies, Red Spider mites and Jassids								
5	Movento	Bayer	Spirotetramat 15.31 % w/w OD	2ml/ ltr	500 ml	500ml	2024	Contact (T)

PESTS OF CHILLIES

1. **Thrips: *Scirtothrips dorsalis* (Thripidae: Thysanoptera)**

- Major pest on peppers throughout tropical Asia.
- Polyphagous, cosmopolitan, found throughout the year and most active in dry weather.
- Both N & A lacerate the tissues and suck the sap from developing leaves
- Young leaves and shoots preferred, however buds, and flowers are also infested.
- Affected leaves curl upwards along the margin and are reduced in size and ultimately shed in severe infestations
- As many as 25 overlapping generations occur in a year.

2. **Black Thrips, *Thrips parvispinus* (Thripidae : Thysanoptera)**

- *Thrips parvispinus* is reported as an invasive pest in 2015 from India.
- Deep punctures and scratches on the underside of the leaves and flowers.
- It resulted in cause large scale shedding of flowers, malformation of fruits and fruit drop in chillies, leading to severe yield loss.
- Insecticides were primarily used by the farmers at an interval of every four days.
- The estimated loss due to thrips damage was 3000- 5000 crores.

***Scirtothrips dorsalis* damage**

***Thrips parvispinus* damage**

Management

- Erection of blue sticky traps @ 25-30 per acre for mass trapping in thrips infested fields.
- Soil application of 200 Kg of Neem cake and 500 Kg of vermicompost per acre to induce resistance against thrips
- Border cropping with 2-3 rows of tall growing crops like sorghum/ maize / bajra / fodder grasses etc. sown thickly as a barrier for thrips movement.

Sl no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Thrips, Aphid, whitefly								
1	Delegate	Dow	Spinetoram 11.7% SC + Neem oil 1ml	1 ml/ lit	250 ml	180 ml	2124	Systemic
2	Movento	Bayer	Spirotetramat 15.31 % w/w OD	2ml/ ltr	500 ml	500ml	2024	Contact (T)
3	Confidor super	Bayer	Imidacloprid 30.5 SC+ Neem oil 2ml	1ml	200 ml	200 ml	595	Systemic

3. **Mite complex**: ***Polyphagotarsonemus latus*, *Hemitarsonemus latus*, *Tarsonemus transluceus*, (Tarsonemidae: Acarina); *Tetranychus cinnabarinus* (Tetranychidae : Acarina)**
   - Mites are white, transparent, very tiny, and not visible to naked eyes. They are polyphagous, cosmopolitan
   - They are found in large numbers on ventral surface of leaves, under a protective cover of fine web. N & A suck cell sap; devitalize the plants causing Murda disease.
   - Mite affected leaves curl downwards and appear twisted and younger leaves get clustered curved down wards.

Sl no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Mites								
1	Applaud	Tata Rallis	Buprofezin 25 % SC	1.5 ml/ ltr	330 ml	1ltr	1320	S & C
2	Intrepid	BASF	Chlorfenapyr 10 % SC	1.5 ml/ ltr	330 ml	100 ml	504	S & C
3	Pegasus	Syngenta	Diafenthiuron 50 % WP	1g/ ltr	240 g	250 g	1023	S & C

4. **Fruit borers: *Helicoverpa armigera; Spodoptera litura; Spodoptera exigua***
(Noctuidae: Lepidoptera)

- Highly polyphagous and cosmopolitan and peak infestation occurs from October to March.
- Colour of the larva varies greatly from yellowish green to green to reddish brown, clothed with sparse hair and have
- The larva initially feeds on the leaves and then on the seeds usually with its head inside the pod and rest of the body outside.

Management:

- Grownup larvae resist most of the available insecticides, so spray contact insecticides in early stages.
- Collection and destruction of egg masses
- Keep Pheromone traps (2/acre) in the field to attract the male moths by which the intensity of pod borers in the environment can be known.
- Poison bait with Rice bran 25 kg + Jaggery 5 kg + Emamectin benzoate 5 SG 125 g/ha (6.25 g a.i. /ha) can be recommended.

Sl no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Borer complex								
1	Exponus	BASF	Broflanilide 20% SC	0.1 ml/ ltr	25 ml	34 ml	2244	Contact (T)
2	Coragen	Dupont	Chlorantraniliprole 18.5 % SC	0.3 ml/ lit	75 ml	60 ml	900	Systemic
3	Proclaim	Syngenta	Emamectin benzoate 05 % SG	0.4g/ ltr	100g	100g	650	Contact (T)

Chemical recommended in chilli ecosystem when a complex of pests present in the field

Sl. no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Fruit borer (*Helicoverpa armigera*), Tobacco caterpillar (*Spodoptera litura*), Thrips (*Thrips tabaci*) and Jassids (*Amrasca devastans*)								
1	Exponus	BASF	Broflanilide 20% SC	0.1 ml/ ltr	25 ml	34 ml	2244	Contact (T)

2	Deligate	Dow	Spinetoram 11.7% SC	1 ml	250 ml	180 ml	2124	Systemic
3	Benevia	FMC	Cyantraniliprole 10.26 % OD	0.5 ml/lit	125 ml	180	1750	Systemic
4	Apex - 50	Crystal	Emamectin Benzoate 1.5% + Fipronil 3.5% SC	1.5 ml/ ltr	250 ml	250 ml	717	S & C
Thrips, Whitefly, Mites								
5	Meothrin	Sumimato	Fenpropathrin 30 % EC	0.5ml/ ltr	125 ml	250 ml	399	Contact (T)
6	Mythri	Coramandal	Fipronil 07 % + Hexythiazox 02 % w/w SC	2 ml	400 ml	1 ltr	2050	Systemic

PESTS OF CUCURBITS

1. Fruit flies: *Bactrocera cucurbitae* (Coquillet) (Tephritidae: Diptera)

The fruit flies are commonest and most destructive pest throughout India. It is found in Pakistan, Myanmar, Malaysia, China, Formosa, Japan, East Africa, Australia and the Hawaiian Islands.

Damage symptoms:

Only the maggots cause damage by feeding on near-ripe fruits, riddling them and polluting the pulp. Damage by the maggots of this pest causes oozing of brown, resinous fluid from fruits and the fruits become distorted and malformed. The maggots feed on the pulp of fruits and cause premature dropping. The attacked fruits decay because of secondary bacterial infection.

Management:

- ❑ Installation of used water bottle baited with cue lure (as MAT) saturated wood blocks (ethanol: cuelure: carbaryl in a ratio 8:1:2) @ 25 traps/ha prior to flower initiation.
- ❑ Use of repellent (NSKE 4%) enhanced trapping and luring in bait spots.
- ❑ Collect infested fruits and dried leaves and dump in deep pits.
- ❑ Frequent rake the soil under the vine or plough the infested field after the crop to kill pupae.

2. Pumpkin beetles: *Aulacophora foveicollis*, *A. cincta*, *A.intermedia* (Galerucidae: Coleoptera)

Both grubs and beetles damage. Grubs remain below the soil surface feeding on roots, underground stems of creepers and on fruits lying in contact with the soil. The adults feed on those parts of the plant which are above the ground. The early sown cucurbits are so severely damaged that they have to be resown.

Management:

- ➢ Early planting of pumpkin during October – November to avoid damage by this pest
- ➢ Frequent raking of soil beneath the crop to expose and kill the eggs and grubs.
- ➢ Hand collection and destruction of infested leaves and fruits.

3. Serpentine Leaf Miner: *Liriomyza trifolii* (Agromyzidae: Diptera)

Damage symptoms: Maggots mines into leaves and cause serpentine mines drying and drooping of leaves.

Sl. no	Trade name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA
Leaf miner (*Liriomyza trifolii*), Red pumpkin beetle (*Aulacophora foveicollis*), Aphids (*Aphis gossypi*), Thrips (*Thrips palmi*), Whitefly (*Bemesia tabaci*), Pumpkin caterpillar (*Diaphania indica*), Fruit fly (*Bactrocera cucurbitae*)								
1	Benevia	FMC	Cyantraniliprole 10.26 % OD	0.5 ml/lit	125 ml	180	1750	Systemic
Fruit borers & Caterpillars								
2	Coragen	Dupont	Chlorantranilipr ole 18.5 % SC	0.3 ml/ lit	75 ml	60 ml	900	Systemic
Whiteflies and Red spider mites								
3	Pegasus	Syngenta	Diafenthiuron 50 % WP	1g/ ltr	240 g	250 g	1023	S & C

PESTS OF CRUCIFERS

1. **Diamond back moth: *Plutella xylostella* (L.) (Plutellidae: Lepidoptera)**

Damage symptoms

First instar larvae mine epidermal surface of leaves producing typical white patches. Larvae, second instar onwards feed externally making holes on the leaves and soil them with excreta. Heavy infestations leave little more than the leaf veins.

Management:

- Raised seed bed
- Mustard as trap crop for every 25 rows of cabbage.
- Use of light traps @ 3/acre for adults of DBM.
- Installation of pheromone traps @ 10/ha for mass trapping
- Light traps are also effective

2. **Leaf webber: *Crocidolomia binotalis* (Pyraustidae: Lepidoptera)**

Young larva feeds gregariously on leaves, later webs together the leaves and feeds. Due to gregarious feeding, rotting of cabbage heads and cauliflower curds are common. Regular pest of minor status but occasionally turn to serious proportions.

3. **Cabbage semilooper: *Trichoplusia ni* (Noctuidae: Lepidoptera)**

Caterpillars start scrapping and feeding on the leaves initially and later defoliate entire plant leaving midribs and main veins. More damage is evidenced in nurseries than in main field.

4. **Cabbage butterfly: *Pieris brassicae* (Pieridae: Lepidoptera)**

The caterpillars alone feed on leaves, young shoots and green pods. When young, they feed gregariously but the grown-up caterpillars migrate from one field to another. Often, entire plants are eaten up.

5. **Cabbage aphid: *Brevicoryne brassicae* (Aphididae: Hemiptera)**

Colonies of aphid are found on tender shoots and suck sap from plant tissues. In case of severe infestation plants may completely dry up and die away.

Sl no	Chemical name	Company	Active ingredient	concentration	Per acre	Packing	cost	MOA

Aphids								
1	Tata Manik	TATA Rallis	Acetamiprid 20 % SP	0.5 g	80 g	100 g	205	Systemic
Diamond back moth (*Plutella xylostella*), tobacco leaf eating caterpillar (*Spodoptera litura)*								
2	Exponus	BASF	Broflanilide 20% SC	0.1 ml/ ltr	25 ml	34 ml	2244	Contact (T)
Cabbage Aphid (*Brevicoryne brassicae*), Mustard Aphid (*Lipaphis erysimi*), Diamond back moth (*Plutella xylostella*), Tobacco caterpillar (*Spodoptera litura*)								
3	Benevia	FMC	Cyantraniliprole 10.26 % OD	0.5 ml/lit	125 ml	180	1750	Systemic
4	Gracia	Godrej	Fluxametamide 10% EC	0.8 ml/ ltr	160ml	100 ml	1250	Contact (T)
5	Keefun	PI industry	Tolfenpyrad 15% EC	2 ml/ ltr	400 ml	500 ml	1500	Contact (T)

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Management of Insect-Pests during Seed Storage and Controlled /Modified Atmospheric methods for safe storage of seeds

Compiled by
Keerthi MC and PVR Reddy
Division of Crop Protection, ICAR- IIHR, Bengaluru- 560089.
Keerthimanikya@gmail.com

Out of the several basic needs of mankind, food is the first and most important one. The world as a whole is in the grip of acute food crisis. In this context the preservation of food grain whatever is produced, is of paramount importance. The problem of feeding the ever increasing human population does not end with production of food grains. It involves a variety of functions right from the protection to deterioration and wastage of all what has been produced unless the problems of storage and that of distribution are satisfactorily solved, the problem of feeding hungry millions may continue even with substantial increase in food production. In this context, the "War on Waste" approach initiated in many countries needs to be stepped up with the improvement of safe storage as the first step in this direction. It is well realized in the grain business that when grain is left unattended for even a short-time, it gets infested with numerous types of tiny insects. Then, if timely measure are not adopted to arrest their multiplication, practically the entire stock is riddled with holes and contaminated with filthy dust, excretion and dead bodies of the insects. Besides insect pests, rats, mice and birds also take a heavy toll of the grain.

Storage insects are direct pests affecting stored grains and causing both quantitative and qualitative loss. Loss means any change in the availability, edibility, wholesomeness or quality of food that prevents it from being consumed by people. Insects cause different kinds of losses *viz*., quantitative loss, qualitative loss, loss of seed viability and damage to storage structure.

3. **Quantitative loss:** By direct feeding insects cause loss in weight of the stored grains. A rice weevil will eat 14 mg out of 20 mg of a rice kernel during its developmental period. But commercially the whole grain is lost. A female weevil, through three generations per year, has the biotic potential to reproduce 1,500,000 off springs which will consume 1,500,00 kernel of rice (amounting 30 kg of rice)

b) Qualitative loss: Storage insects are the main source of quality deterioration by direct feeding on the grain, chemical changes in grain content (nutritive value) contamination of grains with moult skin and body parts and by spreading the pathogenic micro-organisms.

c) Loss of seed viability: When the produce is meant for seed purpose the worth of loss is still more. Insects were found to cause the loss of viability of seeds to an extent of 3.6 to 41% in various crops.

d) <u>Damage to storage structures</u>: Insects like lesser grain borer has the capability to destroy the wooden storage structures, containers and polythene lined bags etc.

The belief that insects outbreak in stored products is spontaneous cannot be true as stored grain insects, originates chiefly from indigenous insect population. The main sources of infestation are field infestation, infection though migration, though conveyance, stored buildings, through birds, ants nest and burrows, survival from last season, infestation of fresh produce, pest build up in store, cross infestation, residual infestation and infestation by flight and crawling insects.

Bio-ecology of storage insect pests:

Stored product insects play a major role in the deterioration of food grains causing both quantitative and qualitative losses. It is well proved that no granaries can be filled with grains without insects as the harvested produce contain egg or larvae or pupae in them because of carryover of filed infestation which cannot be avoided in developing countries like India. Often the presence of the insects in store houses are felt only when they are hovering and flying around, by which time enormous loss and population buildup of insects might have occurred. More than 500 species of insects are associated with stored grains. 100 species cause economic loss. Of these less than a dozen are important pests. The rest are of minor importance or mould eaters or are parasites and predators of pest species. Minor pests constitute approximately 175 species of insects and mites that may rise to population that are injurious to grain and grain products. The group of insects that damage stored grain can broadly be placed in two groups, as 1) Internal feeders, and 2) External feeders of grain or on the finished products of grain. Other way of classification is 1) Primary pests and 2) Secondary pests. Primary pest: pest those damage sound grains and secondary pests are those damage broken or already damaged grains.

4. INTERNAL FEEDERS

Common name	Systematic position	Hosts	Symptoms
Rice weevil	*Sitophilus oryzae S. granaries S. zeamais* (Curculionidae:Coleoptera)	Rice, Wheat, sorghum, barley, maize etc.	Both grub and adults cause the damage. Grains are hollowed out; kernels are reduced to mere powder.
Lesser grain borer	*Rhyzopertha dominica* (Bostrychidae: Coleoptera)	Rice, wheat, maize paddy *etc*	Grubs eat their way into the grain or feed on the grain dust or starchy material and are capable of attacking grain externally. Heating is very common.
Pulse beetle	*Callosobruchus chinesis, C. maculates,* (Bruchidae: Coleoptera)	Pulses, bean and gram.	Grubs eat up the grain kernel and make a cavity. Adults come out making exit holes.
Cigarette beetle	*Lasioderma serricorne,* (Anobiidae: Coleoptera)	Tobacco, wheat, Peanut, cocoa, bean cotton seed	Presence of circular pin head sized bore holes on processed tobacco is the typical symptom of attack. It also attacks the grain of wheat, peanut, cocoa, bean, cotton seed etc.

Drug store beetle	*Stegobium paniceum,* (Anobiidae: Coleoptera)	Ginger, dry vegetables and animal matters	Circular pin-head sized bore hole on turmeric, coriander, ginger, dry vegetable and animal matter.
Angoumois grain moth	*Sitotroga cerealella,* (Gelechiidae: Lepidoptera)	Paddy, Maize and wheat	Only the larvae cause damage by feeding on the grain kernels before harvest and also in store. The larva bores into grain and feeds on its contents. It imparts unhealthy appearance and smell.
Potato tuber moth	*Phthorimaea operculella* (Gelechiidae: Lepidoptera)	Potato, tomato, tobacco, eggplant and *Beta vulgaris*	Damage occurs both in field and storage. Tubers bored by caterpillars associated with fungal or bacterial infection
Sweet Potato weevil	*Cylas formicarius*, (Apionidae: Coleoptera)	Sweet potato, few other species of *Ipomoea*	Grubs and adults bore into the tubers and make them unfit for consumption. Damage occurs both in field and storage

5. EXTERNAL FEEDERS

Common name	Systematic position	Hosts	Symptoms
Khapra beetle	*Trogoderma granarium* (Dermestidae: Coleoptera)	Cereals and groundnut pulses species and pulses cakes	The grubs eat the grain near the embryo or at any other weak point and from there proceed inwards. They can reduce the grain to a mere frass.
Red flour beetle	*Tribolium castaneum, T. confusum,* (Tenebrionidae: Coleoptera)	Maize wheat, etc.	Both the larvae and adults cause damage. The larvae are always found hidden in the food. The adults, however, are active creatures, but mostly found concealed in flour. Adult construct tunnels as they move through flour and other granular food products.
Saw toothed grain beetle	*Oryzaephilus surinamensis,* (Silvanidae: Coleoptera)	Dry fruits, rice, wheat, maize cereals and oilseeds	It feed on grains, dried fruits etc by scarving of grain surface or burrowing holes in them. It attacks rice, wheat, maize, cereal products, oil seeds and dry fruits.
Rice moth	*Corcyra cephalonica*, (Galleridae: Lepidoptera)	Cereals, oilseeds, nuts, dry fruits, rice and pulse	The larvae alone damage the grains of rice and maize by feeding under silken webs. When infestation is high, the entire stock of grains may be converted into a webbed mass.
Tropical	*Ephestia cautella* (Hb).	Wheat, rice,	The caterpillars make tunnels in

Warehouse Moth	(Phycitiae: Lepidoptera)	maize sorghum, groundnut and spices	the food materials. The number of silken tube is sometimes extremely high and these clog the mill machinery where the infested grains have been sent for milling.
Indian Meal Moth	*Plodia interpunctella* (Phycitiae: Lepidoptera)	Maize cereals dry fruits, groundnut, and cereals products	Only the larva causes damage. Crawling caterpillars completely web over the surface of a heap of grains with silken threads.
Grain mite	*Acarus siro* (L.) (Order: Astigmata)	Cereals grains, flour and other eatables	It feeds on the surface of the grains. It attacks cereal grains, flour and other eatables.

INTEGRATED MANAGEMENT OF STORED PRODUCE PESTS

The control methods of stored produce pests can be categorized into preventive and curative measures.

Preventive measures:

- Brush the cracks, crevices and corners to remove all debris in the godown.
- Clean and maintain the threshing floor/yard free from insect infection and away from the vicinity of villages.
- Clean the machines like harvester and thresher before their use.
- Made the trucks, trolleys or bullock carts free from infestation.
- Clean the godown/ storage structures before storing the newly harvested crop to eliminate various bio stages of pest hiding.
- Provide a metal sheet up to a height of 25 cm at the bottom of the wood in doors to arrest the entry of rats.
- Fix up wire meshes to windows, ventilators, gutters, drains etc., to prevent entry of rats, birds and squirrels.
- Remove and destroy dirt, rubbish, sweepings and webbings *etc* from the stores.
- Close all the rat burrows found in godown with a mixture of broken glass pieces and mud plastered with mud/ cement.
- Plaster the cracks, crevices, and holes found on walls, and floors with mud or cement and white wash the stores before storing of grains.
- Provide dunnage leaving gangway or alleyway of 0.75 to 1 m all around to maintain good storage condition.
- Store the food grains in rat and moisture proof storage structures.
- Disinfest the storage structures receptacles by spraying Malathion 50 EC @ 3 lit 100 m^2 before their use.

Curative measures:

i) Ecological methods

- Manipulate the ecological factors like temperature, moisture content and oxygen through design and construction of storage structures/ godown and storage to create ecological conditions unfavorable for attack by insects.
- Temperature above 42 ^{0}C and below 15 ^{0}C retards reproduction and development of insect while prolonged temperature above 45 ^{0}C and below 10 ^{0}C may kill the insects.
- Dry the produce to have moisture content below 10% to prevent the buildup of pests.

- Kill the pests bio stages harbored in the storage bags, bins etc., by drying in the sun light.
- Store the grains at around 10 % moisture content to escape from the insects attack.
- Manipulate and reduce oxygen level by 1% to increase the CO2 level automatically, which will be lethal to all the stages of insects.

ii) Physical methods

- Provide a super heating system by infrared heaters in the flour mills and food processing plants to obtain effective control of pests since mostly the stored produce insects die at 55 –60 ^{0}C in 10 – 20 minutes.
- Modify the storage atmosphere to generate low oxygen (2.4% and to develop high carbon di oxide (9.0 – 9.5) by adding CO2 to control the insects.
- Seed purpose: Mix 1 kg of activated kaolin or Malathion 5 D for every 100 kg of seed and store/pack in gunny or polythene lined bags.
- Grain purpose: Mix 1 kg activated kaolin for every 100 kg of grain and store. To protect the pulse grains, mix activated kaolin at the above dosage or any one of the edible oils at 1 kg for every 100 kg of grain or mix 1 kg of neem seed kernel for every 100 kg of cereal / pulse and store.
- Do not mix synthetic insecticides with grains meant for consumption.

iii) Cultural methods

Split and store pulses to escape from the attack by pulse beetle since it prefers to attack whole pulses and not split ones.

Store the food grains in air tight sealed structures to prevent the infestation by insects.

iv) Mechanical methods

- Sieve and remove all broken grains to eliminate the condition which favours storage pests.
- Stitch all torn out bags before filling the grains.

v) Chemical methods

- Treat the walls, dunnage materials and ceilings of empty godown with Deltamethrin 2.5% WP at 40g / liter at 3 liter for 100 m^2 area in an interval of 3 months.
- Do not spray the insecticides directly on food grains.
- Use seed protectants like pyrethrum dust, carbaryl dust to mix with grains meant for seed purposes only.
- Decide the need for shed fumigation based on the intensity of infestation.
- Check the black polythene sheets or rubberized aluminium covers for holes and get them ready for fumigation.

FUMIGATION

- Use fumigants aluminium phosphide (ALP) to control stored produce pests effectively. Apply aluminum phosphide @ 3 tablets (3 gram each) per 31one of food grains lot with help of an applicator.
- Choose the fumigant and work out the requirement based on the following guidelines.
- 3 tablets of aluminum phosphide 3 g each per 31one of grain.
- 21 tablets of aluminium phosphide 3 g each for 28 cubic meters

- Period of fumigation is 5 days Mix clay or red earth with water and make it into a paste form and keep it ready for plastering all-round the fumigation cover or keep ready sand snakes.
- Place the required number of aluminium phosphide tablets in between the bags in different layer.
- Cover the bags immediately with fumigation cover. Plaster the edges of cover all round with wet red earth or clay plaster or weigh down with sand snakes to make leaf proof.
- Keep the bags for a period of 5-7 days under fumigation based on fumigant chosen.
- Remove the mud plaster after specified fumigation periods and lift cover in the corner to allow the residual gas to escape. Lift the cover after few hours to allow aeration.

Aluminium Phosphide tablets can be used for fumigation.

- Curative treatment is undertaken when classification of food grain stored fall under "Few" of "Heavy" class.
- Aluminium phosphide (ALP) gives out phosphine gas along with Carbon dioxide and Ammonia which are also lethal to pest population besides controlling the inflammable property of phosphine gas.
- Each tablet of 3 grams gives out about 1 gram of phosphine to act as antirespiratory poison.
- The doses of ALP tablets currently used in FCI covered godowns (bag storage) is 9 gm (3 gm × 3 tablets) per MT of food grain.

Sl. No	**Storage type / Nature of fumigation /infestation**	**Dosages**	**Remarks**
1	Covered godown	9 gm/ Tonne	Exposure period 5- 8 days
2	CAP storage	9 gm/ Tonne + 20% extra dose	
3	Shed fumigation	63 gm/ 28 m^3	
4	Khapra beetle infestation	9 gm/ MT + 50% extra dose	

- Usually 50% ALP tablets are spread on the top of the stack while remaining 50% are kept on all sides of the stack to ensure that phosphine gas liberated from ALP tablets is distributed throughout the fumigation system and its concentration gets built up higher than the entrapped air concentration to achieve the lethal doses to kill the entire living pest population.
- The exposure period for ALP fumigation is 5 to 8 days. Exposure for a longer duration will give a better result. Achievement and maintenance of concentration of the fumigant for the required period throughout the system will help in getting cent percent mortality of pest population.
- To achieve and maintain of concentration of the fumigant, the stacks under fumigation must be made air tight by the mud plastering /placement of 'sand snakes' (double row) to retain the phosphine liberated to the maximum extent.
- Also fumigation covers (CLTF covers) under use must be completely free from holes and should not be cut or torn. Complete air tight condition is the essence of success of fumigation operation.

- After fumigation, the stacks should be cleaned/ brushed to remove the residual powder of aluminium hydroxide.
- After exposure period, the stocks should be aerated for some time before issue/dispatch.

New concept in storage pest management:

Controlled atmosphere disinfestations technology involves the alteration of natural storage gases. i.e. CO_2, O_2 and N_2 so as to prevent multiplication of insects, mould growth and quality deterioration of the food grains. The controlled storage atmosphere may be achieved in several ways (Benks and Annis, 1990). About 9.0 to 9.5% CO_2 in air is lethal to all insects. Such atmosphere is called "high CO_2. (Table-14) Low oxygen atmosphere (2 to 4%) generated by purging the pure N_2 and from other sources is called "Low O_2". Allow metabolic processes within storage to remove O_2, usually associated with release of CO_2 i.e. in storage O_2 is consumed by grains and insects during respiration and CO_2 is produced. Thus O_2 level will reduce below 1% and CO_2 will automatically increase which will be lethal to all stages of insect. Such atmosphere is referred as "hermetic storage". Controlled atmosphere technology for control of stored grain pests has been extensively used in the field.

Hermetic storage

The concept of creating a barrier between the commodity and grain has led to the knowledge of hermetic seals. As many conventional structures are internally oxygenated, the idea of de-oxygenation inside the storage structure is utilized in airtight storage to reduce aerobic conditions. This reduction in oxygen (O_2) concentration elevates the carbon dioxide (CO_2), which negatively affects the metabolic activity of the insects and eventually leads to death. Storing grains in hermetic seal storages is an emerging field of engineering research related to agriculture. Storage pest management by an air tight seal or gas-tight seal was advantageous because it increases grain temperature, desiccation, and avoids immigration by enclosing the volatiles inside. Conversely, the gas-tight condition was also detrimental to stored grains. However, in modern management practices, controlled aeration, altering the gas proportion, or including an insect-proof barrier provides additional advantages and success with hermetic storage structures.

Several scientific studies on designing and developing various configurationally modified hermetic storages are available. Compared to bags, they are expensive when designed with metals. Still, they are promoted in the world for modern storage facilities. In Asian and African regions, improved underground storage structures for grains, pulses, and oilseeds were still used, as lower O2 concentration reports desired mortality of insects. A few of the commonest forms of hermetic structures available are silos (metal, cemented, and plastic), metal drums, hermetic cocoons (from few kilos to tons), hermetic bags, and other polymer bags and containers. These hermetic structures are available in different capacities and sizes. Many researchers studied the comparative efficacy of developed hermetic bags. More recently, Yewle et al. (2022) compared the efficiency of a few hermetic bags such as PICS R©, Grain Pro R©, Save Grain R© and Ecotact bags R in comparison with conventional bags such as jute and PP woven. They reported the O2 reduction to 6.4% that results in killing of insects within 2 months of storage. In turn, the grain quality was intact till 6 months.

The following bags may be recommended for long storage of seeds

PICS Bag:

- Triple layer bags consist of two gas-proof inner bags, one inside the other. For protection against physical damage these are placed inside a much tougher, open weave polypropylene (PP) bag. After filling seeds they are tied with string. The inner bags are gas-tight (hermetic).
- Modified atmosphere is created that will kill insect pests. Triple bags usually have a capacity of 50 or 100 kg. The name PICS bag is an acronym based on 'purdue improved crop storage' which is developed from Perdue University, West Yafayette, USA.

Grain pro bag:

- PCI (Pest control of India) recently introduced a hermetic (air-tight) storage system in India in association with grain pro®, Inc. USA.
- This storage system can be used for medium and long term storage needs and eliminate the use of fumigants or chemicals.
- It provide protection against external elements including dust, moisture and other adverse atmospheric conditions during storage and transportation process of various agricultural commodities including seeds.

Zero fly bag:

- First insecticide incorporated storage bag to prevent pest infestations. Without having hazards associated with fumigation
- Active ingredient, deltamethrin, is a FAO and WHO approved insecticide for controlling insect pests. It is incorporated into an individual yarns and slowly released onto the surface of the material in a controlled and sustained manner.
- The commodities stored in the bags are therefore continuously protected against insect infestation for the lifetime of the product

PICS Bag

Grain Pro bag

Zero fly bag

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Assessment of Physiological Maturity in Vegetable Crops

Rajesh Kumar
ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh

Introduction

Time of crop harvest is one of the most important strategy that influences yield, quality and storage of crop.Premature or delayed harvest often adversely affects quality of the produce.Pre-mature or immature harvest is desirable for certain products that are preferred juicy and succulent. Produce may become more fibrous and tough or hard if harvested at full maturity, as in the case of pulse crops for vegetable purpose.There are different stages for various crops, which are demarked as maturity stages; and harvesting in those stages gives high income and good quality produce.Maturity is the stage of fully developed tissues of fruit and vegetables only after which it will ripen normally. During maturation, the fruit receives a regular supply of food material from the plant. Upon maturation, the abscission or corky layer formed at the stem end, stops this inflow of food materials.Afterwards, the fruit depend on its own reserves, carbohydrates are dehydrated and sugars accumulate.

- **Horticultural maturity**
  It is a developmental stage of the fruit on the tree, which will result in a satisfactory product after harvest.

- **Physiological maturity**
  It refers to the stage in the development of the vegetables when maximum growth and maturation has occurred. It is usually associated with full ripening in the fruits. The physiological mature stage is followed by senescence.

- **Commercial maturity**
  It is the state of plant organ required by a market. It commonly bears little relation to Physiological maturity and may occur at any stage during development stage.

- **Physiological Maturity**
  Crop is considered to be physiologically mature when the translocation of photosynthates is stopped to economic part. It refers to a developmental stage after which no further increase in dry matter in the economic part.

Difference between seed and crop production

Seed production	Crop production
Seed plot should be selected carefully for better performance, as per edaphic and environmental	Can be grown in any area

requirement	
Needs isolation from other varieties	Isolation is not necessary
Needs technical skill for maintenance of quality	Special technical skill is not required
Roguing is compulsorily practiced	Roguing is not practiced
Harvesting should be done at physiological/ harvestable maturity	Harvested at field/commercial maturity
Resultant seed should be vigorous and viable	Question of viability does not arise

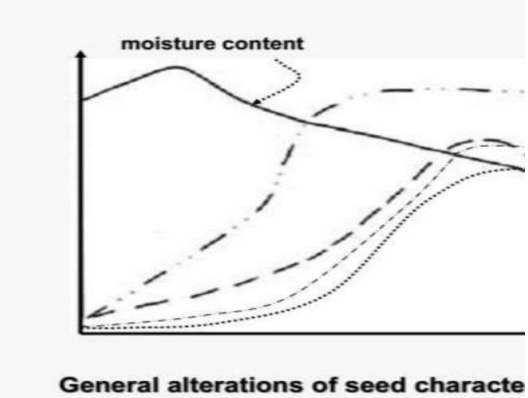
Types of material to be harvested

The types of vegetable seed material to be harvested can be broadly classified into three groups-

- Dry seeds (e.g. brassicas, legumes and onion).
- Fleshy fruits which are usually dried before seed extraction (e.g. chillies and okra.
- Wet fleshy fruits (e.g. cucumbers, melons and tomatoes)

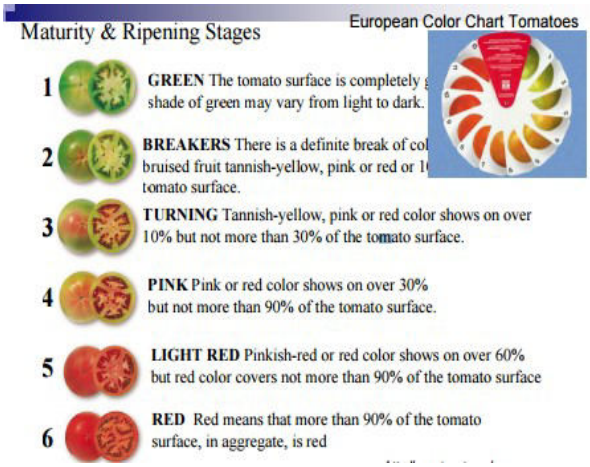
Loss of seeds before and during harvesting

- Shattering, birds, lodging, vulnerability of seed-laden mother plant from weather conditions or other hazards.
- Lodging, possible effects on seed yield and quality.
- Effect of exact harvest stage.
- Balance between immature seeds yet to ripen against subsequent loss, and reduction in yield and quality to achieve maximum yield of high quality seed.
- Effect of weather on yield and quality.



Tomato

- Harvesting is done once the fruits are physiologically mature and turns from green colour to orange or red.
- The fruits that should be harvested are those that are ripe just beyond the eating stage.
- Physiological maturity is the stage of development when maximum growth and maturation has occurred in tomatoes.
- There are six maturity stages viz. mature green, breaker, turning, pink, and light red.



Ripening tomatoes

Ripened tomatoes

Chillies

Chilli seed crops should be allowed for proper maturity.Later in the season, fertilizer applications should be stopped and irrigation should be reduced. This will stimulate fruit to ripen and partially dry the fruit to a leather like condition. Harvesting only red - ripe chilli is done for seed purposes. Immature, green and diseased fruits should be removed completely. Seeds from immature peppers lack vigour and germinate poorly.Seeds were harvested at different maturation stages from 10 to 90 days after anthesis. Seed quality was assessed according to water content, dry weight, germinability, electrolyte leakage, content of starch, neutral lipids, soluble proteins, total soluble sugars, non-reducing sugars, and total free amino acids.

Physiological maturity was reached at 40 days after anthesis, when seeds displayed maximal dry weight and 60% of water loss; however, harvesting maturity was established 50 days after anthesis, taking into account maximal germinability, minimal electrolyte leakage, reserve deposition, and non-reducing sugar accumulation.

Brinjal

- ✓ In brinjal, fruits are allowed to mature beyond the edible stage for seed purpose.
- ✓ The physiological maturity of the fruits is identified by change in colour.
- ✓ The mature fruits of different varieties will vary in colour from yellow to dull purple to brown.
- ✓ The matured fruits are harvested by hand picking and hung in sheds until their colour dulls.

Okra

- The physiological maturity of pods is identified by a change in colour from green to brown and by the drying of the pods.
- Pods should be harvested at the right time, since dried pods tend to dehisce (split open) with very little force.

Cucurbitaceous crops

- Harvesting fruit for the purpose of seed extraction in cucurbits is

normally done when the fruits are ripe, however, it can be performed even before complete maturation, and followed by post-harvest storage

- For cucurbits, the process of seed maturation continues after harvest
- It reaching maximum levels of germination and vigor after undergoing a rest period, which varies among different species

Table: Crop maturity stage for seed purpose

Crop	Maturity symptoms
Cucumber	Dark brown fruits
Bitter gourd	Fully mature fruit of yellow colour
Round melon	Mature fruits
Musk melon	Fruit detachment from dried stem
Watermelon	Dryness of vine, dull sound of fruits, yellowness of fruits touching soil surface
Pumpkin	Colour change – yellowing- drying of fruits
Sponge gourd	Brown dry fruits
Bottle gourd	Colour change –pale green to whitish- drying fruits
Pumpkin	Complete yellowing

Pumpkin

The physiological maturity can be identified by colour change from green to yellow and drying of the fruit stalks. The matured fruits should be harvested by hand picking and stored for few weeks for further maturation of seeds. Pumpkin fruits can be harvested at 55-65 DAA for obtaining seeds with maximum physiological quality. Pumpkin seeds reach physiological maturity in the period between 55 and 65 DAA, when they have the lowest water content and electrical conductivity practically stable. After harvest, the storage of fruits for a period of 15-20 days is essential to ensure seed quality.

Cowpea

- The pod length reaches maximum at 12 days after anthesis in both cowpea varieties (bush and pole type) with higher pod weight and hence it is considered as the optimum stage of harvest for vegetable purpose.
- Seed development and maturity takes place in the second phase.
- Seed qualities (germination and vigour) were maximum in pods harvested at 16 days after anthesis in bush cowpea variety "Bhagyalakshmi" and at 18 days after anthesis in pole cowpea variety "Lola" and hence considered as the optimum physiological maturity stage for seed purpose harvesting.

TABLE 2. Seed characteristics of cowpea varieties at different growth stages.

Days after anthesis	Fresh seed weight/pod (g)		Dry seed weight/pod (g)		Germination %		Vigour index	
	Bhagyalakshmi	Lola	Bhagyalakshmi	Lola	Bhagyalakshmi	Lola	Bhagyalakshmi	Lola
2	0	0.00	0	0.00	0	0.00	0	0.00
4	0	0.00	0	0.00	0	0.00	0	0.00
6	0	0.20	0	0.02	0	0.00	0	0.00
8	0.15	0.45	0.01	0.03	25.6	0.00	163.7	0.00
10	2.2	2.77	0.2	0.37	60.1	0.00	419.8	0.00
12	2.9	4.50	0.2	0.77	69.3	44.63	756.7	551.47
14	5.1	5.17	1.5	1.20	80.1	69.30	948.2	848.63
16	3.9	7.42	1.6	2.40	92.6	83.70	1198.9	1510.00
18	3.3	8.33	1.6	2.63	90.2	88.00	1043.2	1552.50
20	2.9	6.58	1.3	2.63	82.1	69.67	947.9	1022.90
CD(0.05)	0.78	1.2	0.1	0..3	7.5	6.3	104.0	89.4

Krishnakumary, 2012. Pattern of fruit and seed development in vegetable cowpea varieties, Legume Research, 35 (1) : 53 – 55.

Onion

- In a study, seed quality in terms of germination and vigour were maximum at 40 and 45th DAA in ArkaBindu.
- The seed attained physiological maturity between at 45th DAA.
- Seed moisture content of 16.6% was found to be the right stage for harvest.

Table 3. Seed quality as influenced by seed developmental stages in onion cv. Arka Bindu

Treatments	Germi-nation (%)*	First count (%)*	Seedling Root length (cm)	Seedling Shoot length (cm)	Seedling Vigour Index	EC (µs/cm)
15 DAA	0.0 (0.0)	0.0 (0.0)	0	0	0	157.7
20 DAA	1.0 (2.9)	0.0 (0.0)	0	0	0	152.7
25 DAA	7.0 (14.9)	2.5 (8.9)	0	0	0	157.6
30 DAA	10.5 (18.8)	4.0 (11.3)	0	0	0	102.8
35 DAA	45.5 (42.3)	14.5 (22.3)	5.2	6.2	518	134.5
40 DAA	95.0 (76.9)	77.0 (61.2)	6.4	8.9	1456	128.6
45 DAA	96.0 (78.4)	83.0 (65.6)	6.7	8.9	1474	97.3
SEm ±	2.5	1.3	0.3	0.3	50	12.0
CD at 5%	5.25	2.73	0.6	0.6	104	25.8
CD at 1%	7.15	3.72	0.8	0.9	141	35.8

* Figures in the paranthesis are arc sine transformed values

Lablab bean

- Pod and seed growth, and seed composition (protein, fat, carbohydrate, ash, total soluble solid, TSS) of two genotypes (DS-52 and DS-106) of Sem (Indian bean) were investigated to assess the stage of physiological maturity, time of harvest of vegetable pod and seed germination at physiological maturity.
- It was found that physiological maturity of seed attained around 40 DAF with standard germination (> 80%) Das and Fakir, 2014.

Peas

- For table purpose, the high quality of pea is associated with tenderness and high sugar content.
- During maturity sugar content decreases rapidly and there is an increase in starch and other polysaccharides and insoluble nitrogenous components such as protein.
- Harvesting of pea seed crop is done when more than 25% pods are ripe and almost all pods are matured.
- Plants are pulled up for curing for 3-5 days. Delay in harvesting may lead to shattering of pods.

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Hybrid seed production in Chilli and Capsicum

Rajesh Kumar
Principal Scientist, IIVR Varanasi (UP)

The genus *Capsicum* consists of approximately 30 species, out of which five species viz., *C. annuum, C. frutescens, C. baccatum, C. chinense* and *C. pubescens* are cultivated for various purposes (Poulos, 1994).The enormous horticultural, agricultural and biological diversity of chilli and sweet pepper (*Capsicum annuum* L.) has helped to make it globally important crop for uses as a fresh and cooked vegetable (e.g. for salads, warm dishes, pickles), a source of food ingredients for sauces/powders and as colorant (Bosland and Votava, 2000). The species are also used for medicinal purposes and provides the ingredient for a non-lethal deterrent or repellent to some human and animal behaviour (Krishna De, 2003). The fruits are rich in vitamins C and A, however, the two chemical groups of greatest interest are the capsaicinoids and the carotenoids. Chillies in a fresh state are very rich in vitamin C (ascorbic acid), as shown by Dr. Szent Gyorgyi, the Hungarian scientist, who was awarded the Nobel Prize in 1937 for isolating vitamin C from paprika fruits showing that they were one of the richest sources available of this vitamin (Anu and Peter, 2000). The capsaicinoids are alkaloids that give hot chilli peppers, their characteristic pungency. The rich supply of carotenoids contributes to chilli peppers' nutritional value and colour (Perez-Galvez *et al*., 2003). The cultivars of *C. annuum* include both hot pepper (pungent fruits) and sweet pepper (non-pungent fruits, commonly called as capsicum). Sweet pepper is often called as bell pepper because majority of sweet pepper cultivars grown worldwide have bell shaped (four lobbed) fruits. In India, *C. annuum* is cultivated for fresh fruits (both sweet & hot types), oleoresin/colour extraction (paprika type) and processing (pickle type) (Kumar *et al*., 2006). History of chilli cultivation in India is very old; however sweet peppers were introduced during British rule.

The major chilli producing countries are India, China, Korea, Hungary, Spain, Nigeria, Thailand, Turkey, Kenya, Sudan, Uganda, Japan, Ethiopia, Indonesia, Pakistan, and Mexico. The world chilli production (green fruits) over the past five years has increased not only due to increase in area under cultivation, but also because of increase in the productivity. According to the FAO (2011) figures, the world production of chilli (both green and dry) during 2009 was 31.2 mt on an area of 3.72 mha, while India produced about 1.36 mt of chillies in an area of 0.807 mha with a productivity of 1.68 t/ha, however, the potential productivity lies between 30–40 t/ha. However, as per the NHB (2010) report, chillies (dry) was grown on 0.77 mha with a production of 1.203 mt along with a productivity of 1.6 t/ha. The major chilli growing states in India are Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu, which together constitute about 75% of the total area. Andhra Pradesh ranks first in dry chilli fruits production followed by Tamil Nadu, Maharashtra, Orissa and Karnataka. Important reasons for the lower yield of Indian chillies could be unavailability of good quality seeds, attack of pests and diseases and climatic vagaries. India has immense potential to export different types of chillies required by various markets around the world. Indian chilli exports are mainly influenced by domestic demand and uneven production, which is interrupted by erratic monsoon, drought and yield factor. The development of hot pepper F_1 hybrid along with resistance to major diseases is one of the most practical, economical and environmentally secure strategies for reducing yield losses.

The use of hybrid seed has become popular among the commercial vegetable growers and farmers despite of higher seed cost. The exploitation and extent of heterosis varies in a number of vegetable crops including chillies. In capsicum, hybridization is performed manually or using the genetic emasculation tools, the former being labour intensive process. This warrants the economical production of hybrid seeds and viable hybrid seed production technology should be developed in order to realize the best potential of hybrid.

Cultural practices

For hybrid seed production in chillies, raising of crop is almost similar to the crop grown for commercial purposes. Briefly, for nursery, a raised bed of 1 m width and length according to availability, soil having fine tilth and well decomposed FYM is mixed thoroughly. Seeds treated with suitable pesticides are sown in rows 5 cm apart at distance of 1 cm. After covering with dried leaves/straws, the bed is watered and subsequently plant protection measures are taken during the nursery growing. At about 30 days after sowing, the plants are ready for transplanting which are transplanted at 60 cm (row-row) x 45 cm (plant-plant) and crop management is followed as for the commercially grown chilli crop.

Hybrid development in chillies

The methodology for hybrid seed development in chilli and sweet pepper is similar. F_1 hybrids have superiority over most of the popularly grown open pollinated cultivars due to their earliness, higher productivity, fruit quality, greater adaptability, uniformity and resistance to biotic and abiotic stresses. Moreover, hybrid/heterosis breeding is comparatively easy to vegetable breeders as it is easy to incorporate resistant genes for biotic/abiotic stresses and also horticultural traits in F_1 hybrid. Besides it also gives the right of the bred variety is protected in terms of parental lines. Chilli peppers express considerable amount of heterosis (20–50%) for yield and thus are easier for exploitation of hybrid vigour as F_1 hybrids.

Chilli peppers grown from hybrid seed are highly uniform and usually higher yielding. In chilli, percent share of hybrid varieties are very low *i.e.* approximately 2.5 to 3 percent. The reason may be probably due to small flower size and low seed yield per an act of pollination. Though chilli is considered as a self-pollinated crop the extent of cross-pollination reported is 60–90 percent, may be due to exerted nature of stigma. Therefore, to maintain purity of the parental lines, a minimum of 400–500 m isolation distance is recommended.

Procedures for the development of F_1 hybrids in chillies

The flowers of chillies are hermaphrodite (both male and female reproductive parts are found in same flower) in nature. The procedures of hybrid seed production described in several books and articles for many other crops are suited for chilli and sweet pepper as well. The steps viz., production of inbred lines, testing of combining ability, improvement of inbred/ parental lines, emasculation & pollination (for manual hybrid seed production) and hybrid seed production are considered for the production of hybrids/hybrid seeds in that order. Moreover, there exist genetic mechanisms for hybrid seed production in chillies and sweet peppers i.e. use of male sterility (genetic as well as cytoplasmic-genetic male sterility) systems.

Hybrid seed production using hand emasculation and pollination

Manually, hybrid seeds in chilli and sweet pepper are produced through hand emasculation and pollination method. This involves, identification of appropriate stage of bud to be pollinated (bud selection), its emasculation and pollination. Generally, the buds which are

closed and may open in following 1-2 days are selected (before anthesis) and the anthers are removed with the help of forceps (emasculation). In the following morning of emasculation, the emasculated buds are pollinated with freshly harvested pollens from the male parent (pollination). Proper labelling of the pollinated flowers is done to facilitate the harvesting of crossed fruit containing F_1 hybrid seeds upon maturity of the fruits. Generally it takes about 30-40 days after successful pollination for maturity of the fruits.

Male sterility in chillies

Among the genetic emasculation tools, both genetic male sterility (GMS) and cytoplasmic-genetic male sterility (CGMS) have been employed in hybrid seed production of chillies. Male sterility is caused due to the absence of functional anthers, pollen or male gametes in otherwise hermaphroditic flowers.

Genetic Male Sterility

Genetic male sterility is one of the means by which hybrid seeds are produced in chillies. The sterile plants are used as the female parent of a hybrid cross. The male sterile characteristic is often inherited as a single recessive gene (*ms*). The use of genetic male sterility is limited in hybrid seed production due to the inefficiency of producing and maintaining a population of male sterile plants. In order to produce more male sterile plants one must cross a fertile plant heterozygous for the male sterile trait to the male sterile plant, and then only half the progeny from this cross will be male sterile, thus the GMS being maintained in heterozygous condition.

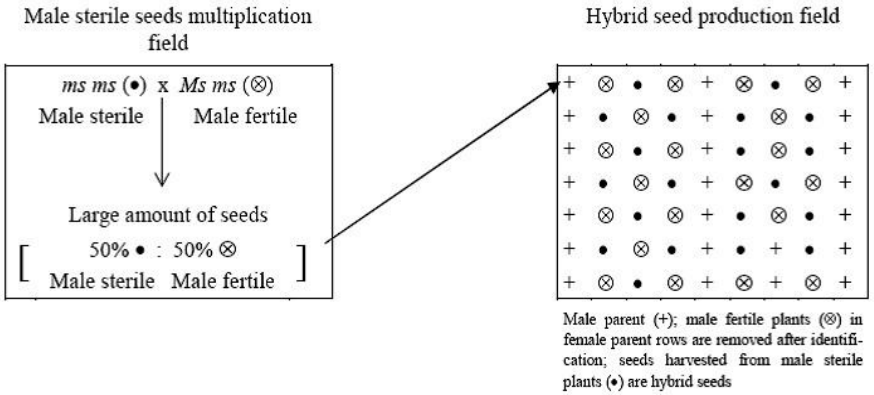

Fig-1. General scheme of hybrid seed production using GMS system

During hybrid seed production, the heterogeneous population consisting of male fertile and male sterile plants and the male parent of interest are planted in alternate rows in the plot in isolation (400-500 m from other commercially grown chilli crop). After flowering one can easily distinguish the sterility/fertility by observing for pollens through nail test (shed pollen from the flower on to the nail and if whitish powder like particles are seen- it indicates male fertility, and if no powdery substance is seen, the plant is male sterile) or through pollen viability test under the microscope. The male fertile plants are removed from the plot and the remaining plants from the heterogeneous population are female fertile (male sterile). The seeds developed on the male sterile plants after open pollination from the male fertile plants (male parent of interest) are the F_1 hybrid seeds.

Cytoplasmic Male sterility

Cytoplasmic-genetic male sterility (commonly called CMS system) is another mean by which hybrids may be produced in chillies. The advantage of a CMS system is that a population of sterile plants can be generated in which all the offspring are sterile. Sterility results from an interaction of nuclear and cytoplasmic genes, hence called cytoplasmic-genetic male sterility. CMS is maternally inherited and is with a specific (mitochondrial) gene whose expression impairs the production of viable pollen. Since restorer of fertility (*Rf*) genes in the nucleus function to suppress the CMS phenotype, nuclear restoration allows commercial exploitation of the CMS system for the production of high yielding and heterotic hybrid seeds and avoids the need for intensive labour and extensive hand emasculation. Dominant restorer alleles have been identified in several hot pepper genotypes, to help breeders to differentiate restorer lines from maintainer lines to secure a completely sterile female parent, but fully fertile hybrids. Since male part (constricted anthers with little pollen) is sterile, use of such line precludes huge cost on manual emasculation. Thus, utilizing CMS line, the production cost of hybrid seeds can be drastically reduced. The CMS line (also called 'A'-line) is maintained by making crosses on it using pollen from maintainer line (also called 'B'-line). Through such crossing, 100 percent male sterile seeds are obtained, unlike GMS line. The seedlings prepared from such crosses can be transplanted as female rows (4) along with the selected restorer line (called 'R'-line) as male row (1) in the hybrid seed production field. Since considerable amount of natural cross-pollination occur in chilli, no hand pollination is required. However, for economic seed yield of 300–350 kg/ha, hand pollination is recommended. Systematic study on honeybee activity in increasing natural seed yield in chilli using CMS lines is required.

CMS in hot pepper plants (*Capsicum annuum*) was first documented by Peterson (1958) in the PI 164835 line introduced from India. Studies using Peterson's CMS material indicate that additional factors affect pollen sterility and stability. This trait was found to be controlled by a major recessive *rf* gene interacting with a specific S-cytoplasm. The S-cytoplasm of this line is usual source of CMS used in the production of hybrid seeds of chilli pepper. Using these male sterile lines, hybrid cultivars (Kashi Surkh from IIVR, Varanasi; Arka Meghana, Arka Sweta and Arka Harita from IIHR, Bangalore) were identified for commercial exploitation.

After completion of hybridization programme, all the non-crossed (untagged) fruits developing/developed through natural cross-pollination (NCP) on female plants are removed, which facilitates vigorous development of crossed fruits and seeds. In case of CMS/GMS based hybrid seed production, this practice is not required, as all the fruits developed on the male sterile plants will be crossed fruits, provided recommended isolation distance is maintained.

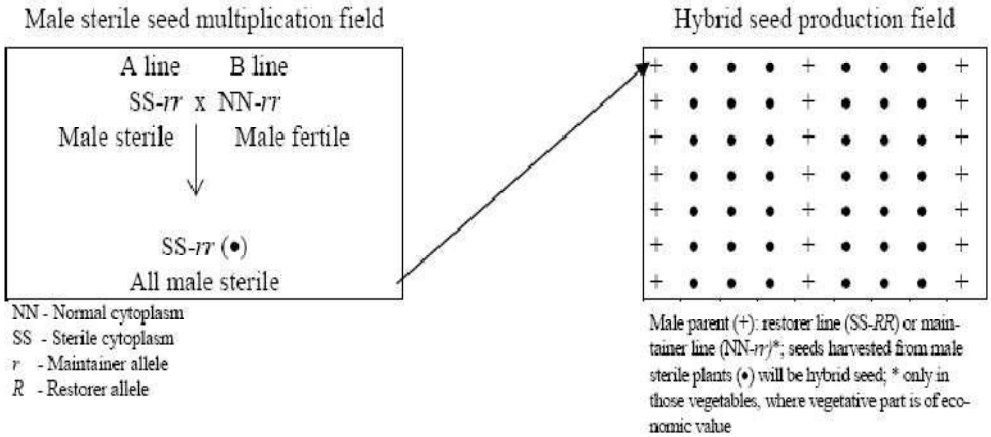

Fig-2. General scheme of hybrid seed production using C-GMS system

The number of crossed fruits per plant should be kept as many as possible. However, 50–70 crossed fruits per plant are optimum for getting a good seed yield of hybrids. It may also depend on the nature and genetic behaviour of the parental lines for fruit bearing habit, plant structure and other intercultural operations.

Harvesting of crossed fruits

Fruits of chilli mature at 30–40 days after pollination. The maturity index is red ripe fruit. Before harvesting of crossed fruits, open pollinated (non-hybrid) fruits are removed in order to eliminate chance contamination in hybrid fruits. Hence it should be secured that only tagged fruits are harvested. In case of CMS or GMS based hybrid seed production, all the fruits developed on CMS or GMS plants will be crossed fruits, provided recommended isolation distance is maintained.

Seed extraction and packaging

The harvested ripe fruits are dried and seeds are separated by maceration (commercial scale) or by longitudinal bifurcating (experimental scale) of the fruits. After extraction, seeds should be dried up to 8 percent moisture level. Before packing in appropriate moisture proof packing material, seeds should be cleaned on density gradient seed cleaner. On an average seed yield of 300–350 kg/ha (with 10–15 g/plant) is obtained in chillies.

Apart from public institutes, many private seed industries are extensively marketing different chilli varieties/hybrids. Some of the popularly grown private chilli hybrids in major chilli growing areas are:

Public institutions		**Private organizations**	
Chilli hybrid	**Source**	**Chilli hybrid**	**Source**
Kashi Surkh (CGMS based)	IIVR, Varanasi	Delhi Hot	Seminis
Kashi Ageti	IIVR, Varanasi	Indam 10	IAHS
CH 1 (GMS based)	PAU, Ludhiana	BSS 378	Bejo Sheetal
CH 3 (GMS based)	PAU, Ludhiana	BSS 275	Bejo Sheetal
Arka Meghana (CGMS based)	IIHR, Bangalore	NS 1101	Namdhari seeds
Arka Sweta (CGMS based)	IIHR, Bangalore	Roshni	Syngenta
Arka Harita (CGMS based)	IIHR, Bangalore	HPH 232	Syngenta
		Tejeswini	MAHYCO seeds
		Soldier	Nunhems seeds
		ARCH 82	Ankur seeds
		ARCH 228	Ankur seeds

Sweet pepper Hybrids

F1 Sweet pepper	**Company**
Orobelle (yellow)	Syngenta
Bomby (red)	Syngenta
Indra (green)	Syngenta
Lario (green)	Syngenta
Mahabharat (green)	Indo-American Hybrid Seeds
Laxmi (red)	Indo-American Hybrid Seeds

Super Gold (yellow)	Indo-American Hybrid Seeds
US 181 (green)	US Agriseeds
Bell Boy (green)	Generic Seeds

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Quality Seed Production of Varieties/Hybrids in Brinjal

Dr.PidigamSaidaiah, Ph.D

Associate Professor, Dept. of Genetics and Plant Breeding, SKL Telangana State Horticultural University, College of Horticulture, Mojerla, Telangana

Climatic requirements: Brinjal is a warm season crop and is susceptible to severe frost. A long and warm growing season is desirable for successful brinjal production.Cool nights and short summers unfavourable. However, late round varieties tolerant to mild frost to some extent than early long varieties. A daily mean temperature of 13 to 21°C is most suitable for better growth and yield. Its seed germinate well at 25°C temperature.

Flower characters: In most Indian varieties flowers are born in clusters. Some varieties particularly Japanese and some American bear flower singly. In both single flower bearing and cluster bearing type, the flowers are perfect. In Pusa Purple Long and Pusa Kranti, the inflorescence may contain 4-5 flowers but only first flower is perfect and rest of the flowers being incomplete drop out. Four types of flower appear in brinjal :

1. Long styled with normal ovary.

2. Medium styled with normal ovary.

3. Short styled with rudimentary ovary.

4. Pseudo-short styled with rudimentary ovary.

It is first type which sets fruit. In case of cluster bearing type e.g. Pusa Purple Cluster, all flowers in the cluster are of first type i.e. long set fruits.

Pollination: In brinjal cone like formation of anthers favour self pollination, in the same way as it occurs in tomatoes. The anther discharge the pollens through terminal pores Since stigma ultimately projects beyond the anthers, therefore, there is ample opportunity for cross pollination because in such position it comes more easily into contact with insect (bumble bees) visiting the flowers. Cros pollination in India under Delhi conditions is 1. to 1.99%, whereas in Bulgaria 30-40% and i Japan 0.2 to 46.8%.Opening of flowers in brinjal starts by 7.0 or 8.00 a.m. depending upon the temperature Lower temperature delays the opening. The opening of flowers continue till 2.00 p.m., after which flowers start closing back which continue Fill late evening. The same flower reopen next day and similar cycle continues for three-four days. The receptivity of stigma is maximum or the day of anthesis and decreases in following days.
To increase the production of long and medium styled flowers, application of more nitrogen or spraying of growth regulators during pre- flowering and flowering stages may be followed.
Method of seed production: Seed to Seed

Stages of seed production: Breeder seed → Foundation Seed I → Foundation Seed II → Certified Seed.

Varieties: According to colour, there are white, yellow, brown, green, pink, black, purple and striped, while according to shape they may be round, Hisar Shy oblong, long and pear shaped. In north India Hisar. This is pinkish purple or violet and black varieties are dark purple preferred.

Varieties: CO.1, CO.2. MDU 1, PKM.1, KKM.1, PLR. 1. AU1, Pusa purple long, Arkanidhi, Pant smart, Arkaneelkanth, Arkashrish.

Hybrids: COBH1, Arka Navneet (IIHR 22 1 x Supreme), Pusa H 5, Pusa H 6.

Season: May- June and December – January

Land requirement: The land should be free of volunteer plants.

Isolation: For varieties, 200m and 100m of isolation distance is required for foundation and certified seed, respectively. For hybrid seed production, minimum of 200 M isolation distance should be maintained.

Seed rate: Varieties – 400 to 500 g/ha. Hybrids – 200 g/ha (Female) and 50 g/ha (Male)

Seed treatment: Seed treatment with *Trichoderma viride* @ 4g Kg^{-1} before sowing can be practiced against the incidence of damping off disease. Drenching of Copper Oxy Chloride at 0.1% at weekly interval minimize this disease.

Nursery: Sow the seeds in raised nursery bed of 20 cm height, in rows of 5 cm gap and covered with sand.Eight and ten nursery beds will be sufficient to transplant one acre. Apply 2 Kg of DAP 10days before pulling out of seedling.

Transplanting: Seedlings are transplanted when they are 30 35 days old (12 15 cm height) preferably in the evening time. Spacing of 75 x 60 cm (non spreading) and 90 x 60 cm (spreading) varieties, 90 x 60 cm for female parent and 60 x 45 cm for male parent of hybrids.

Manuring: The field should be thoroughly ploughed for fine tilth and apply 25 tons of FYM/ha. The other fertilizer requirement for brinjal variety and hybrid are same as followed for tomato seed production.

Roguing: The 47ouging should be done based on the plant characters, leaf, branching and spreading characters and also based on fruit size, shape and color. The plants affected by phomopsis blight, leaf spot and little leaf virus disease should be removed from the seed production field.

Specific Field Standards:

Factors	Foundation	Certified
Off types – Variety (max)	0.1%	0.2%
Off types – Hybrid (max)	0.01%	0.05%
Designated diseased plant (max)	0.1%	0.5%

The designated diseases in brinjal are Phomopsis blight caused by Phomopsis vexans and little leaf caused by Datura virus 2.

Specific requirements

Factor	Maximum permitted %	
	Foundation	Certified
*Offtypes	0.10	0.20
**Plants affected by seed borne diseases	0.01	0.50

* Maximum permitted at and after flowering in the case of offtypes and at the final inspection in the case of seed borne diseases.

**Seed borne diseases shall be: Phomopsis blight (*Phonopsisvexans*)

Pest and disease management

Epilachna beetle	Damping off disease
Whitefly	Damping off
Shoot and fruit borer	Leaf Spot
Ash Weevil	Little Leaf
Aphid	Nematode
Red Spider mite	

Seed storage

Seeds obtained from the first picking stored well for a longer time than those obtained from fifth and sixth pickings. The rate of deterioration was also faster in seed obtained from the later pickings. The seeds stored in PAFP pouches recorded higher germination for thirty months after storage as compared those in cloth bags.

Seed Yield:100 to 200 kg/ha

Seed Standards (Variety & Hybrid)

Factors	Foundation	Certified
Pure seed (mini)	98%	98%
Inert matter (maxi)	2%	2%
Other crop seeds (maxi) no/kg	5/kg	10/kg
Weed seeds (maxi) no/kg	5/kg	10/kg
Germination (mini)	60%	60%
Moisture (maxi)	8%	8%
For VP container (maxi)	6%	6%

Hybrid seed production

The planting ratio of female and male parents adopted for hybrid seed production is normally 5:1 or 6:1.For production of hybrid seeds, crossing programme is done using emasculation and dusting methods as followed in tomato.

Emasculation and pollination: Emasculation is done in the afternoon hours. Of the four types of flowers, only the flower buds having long or medium style are emasculated. In brinjal,

flowers appear both as solitary or in cluster, in the noncluster fruited cultivars, generally single flower in a cluster of 3-5 is long or medium styled. After selecting the long or medium styled buds, the corolla is opened gently from the side, length of the style is glanced and then the anthers are picked off separately by upward pull with the forceps. The emasculated flowers are protected by thin cotton wool.

Using a needle, the anther cone is carefully removed without disturbing the style. These emasculated flowers have to be covered by butter paper bags. Similarly the flowers of male parent from which pollen is going to be collected have to be bagged on the previous day evening. Next day morning by 6 am the flower buds are collected before opening. Anthers are separated and kept in petridish covered by glass. These petridishes are kept against sunlight to facilitate dehiscence of anthers and release of pollen grains. The butter paper cover on the emasculated flower is removed and the pollen is transferred to the stigmatic surface with the help of a camel hair brush or by smearing the dehisced anther on the stigma. A part of sepals of the pollinated flowers are cut with the help of a scissors for identification mark that they are hybridized flowers. After hand pollination these flowers are rebagged. Since the stigma is receptive for 4 days, the bags should be removed after about 8-10 days only when the fertilized ovary will prominently seen. After full maturity and ripening of the fruits, seeds are extracted. On an average a quantity of 400 kg of hybrid seeds can be obtained from one hectare.

Male sterile lines: Use of male sterile lines reduces the cost of seed production. In brinjal functional male sterile mutants was also reported. This is at a recessive character determined by single gene.

Harvesting and processing: Harvesting is done when fruits are fully ripe (when the fruits turn into yellow colour) *i.e.*, 45 days after flowering. The harvested fruits are to be graded for true to type and off type and fruit borer infested fruits are discarded. The graded fruits are cut in 2 3 pieces or whole fruits will be put in a cement tank with water and crushed manually and then allow it for fermentation for 1 2 days. Then the floating pulp portions are to be removed, the seeds settled at the bottom should be collected and washed with water and then the seeds are treated with commercial Hydrochloric acid @ 3 5 ml/kg of seed. The mixture is kept for 10 -15 minutes with frequent stirring. Then the treated seeds are to be washed with water for 3 4 times. Afterwards seeds are dried under shade for 2 3 days over a tarpaulin and followed by sun drying for 1 2 days to reduce the seed moisture content to 8 per cent. Then the seeds are cleaned and graded with BSS 12 sieve. The processed seeds are treated with fungicides or Halogen mixture @ 5g/kg of seed.

To upgrade the seed lot water floatation technique and specific gravity grading are commonly used. Seeds can be stored in aluminium foil pouches by which the viability can be maintained upto 18 months under ambient condition, by dressing the seeds with 2 g of thiram / kg of seed.

Seed treatment: Seeds must be treated with fungicides before storage. The seeds may be treated with Captan or Thiram @ 4g/kg of seeds. The seeds can also be treated with halogen mixture @ 5g/kg of seed and it is an ecofriendly seed treatment.

Storage containers: Seeds could absorb moisture from atmosphere. Hence storing seeds in the coastal region (or) river sides we should use moisture vapour proof containers *i.e.* 700 gauge polyethylene bags. For seed storage every time new containers must be used.

Seed Yield:100-200 Kg/ha

Seed Standards (Variety & Hybrid)

Factors	Foundation & Certified
Pure seed (mini)	98%
Inert matter (maxi)	2%
Other crop seeds (maxi) no/kg	None
Weed seeds (maxi)	None
Germination (mini)	70%
Moisture (maxi)	8%
For VP container (maxi)	6%
Genetic purity required for brinjal hybrids	90%

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Quality Seed Production of Varieties/Hybrids in Tomato

Dr.PidigamSaidaiah, Ph.D
Associate Professor, Dept. of Genetics and Plant Breeding, SKL Telangana State Horticultural University, College of Horticulture, Mojerla, Telangana

Climate: Tomato is a warm season crop and requires a frost free period of about four months for seed production. Optimum temperature for seed germination is 16° to 29°C and for growth and fruit set between 20° to 25°C. Below 15°C and at/above 32°C, the pollen germination is very poor. Hence, very high or very low temperature and draught adversely affect fruit setting. High temperature combined with dry winds causes blossom drop. A warm and sunny weather is most suited for proper fruit set, fruit and seed development which results in higher seed yield.Tomato is a typical day neutral plant. It requires temperature of 15-20° C for fruit setting.

Flower characters and pollination: The number of flowers vary from 4 to 12 in each cluster, average number is 4-5 per cluster. The flowers are small pendent, calyx are 5-6, sepals parted green in colour with many hairs. This persist until fruit mature. Corolla sulphur yellow in colour and form a short tube. Stamens five in number and are borne on the throat of the corolla, long anthers partly united. Pistil two or multicarpellary, elongated style, simple bulbous stigma, stigma extend through and somewhat beyond encircling androecious.

Daily temperature influences time of opening of flower, anther dehiscence and stigma receptivity. Anthesis is correlated with temperature and soil moisture. The petals open between 8-10.30 a.m. The dehiscence takes place between 9-10.30 a.m. The receptivity of stigma is between 8.30 to 11.30 a.m. The dehiscence is 24 to 48 hours earlier than opening of corolla, hence tomato is a self pollinated crop. Bumble bees sometimes visit tomato flowers and cause cross pollination. About 1.7 to 3.8% cross pollination has been T reported under Delhi conditions.
Method of seed production: Seed to Seed.

Stages of seed production: Tomato is a self-pollinated crop, hence either three or four generation model could be adopted.

Open Pollinated Varieties: Breeder seed → Foundation Seed → Certified Seed
Breeder seed → Foundation Seed I → Foundation Seed II →Certified Seed

Indeterminate varieties: Pusa Ruby, Solan Gola, Yaswant (A 2), Sioux, Marglobe, Naveen, Ptom 9301, Shalimar 1, Shalimar 2. Angurlata, Solan Bajr, Solan Sagun, Arka Vikas and ArkaSaurbh.

Determinate varieties: Roma (EC 13513), Rupali, MTH 15, Ptom 18, VL 1, VL 2, HS 101, HS 102, HS 110, Pusa Early Dwarf, Pusa Sheetal, Floradade, ArkaMeghli, CO.1, CO.2, CO.3 (Marutham), PKM.1, Py1,

Hybrids:
Breeder seed → Foundation Seed → (Multiplication of parental lines) Certified Seed (Production of F1 hybrids).

Hybrid varieties:COTH 1, 2 and 3 Pant Hybrid 2, Pant Hybrid 10, Kt 4. Pusa Hybrid l 4, ArkaShreshta, Arka Vardan, Arka Abhijit, Navell 1 &2 (Sandoz), Rupali, Sonali, MTH 6

Season: It is highly suitable both for *Kharif*(May – June) and *Rabi*season (November - December)

Land requirement: The previous crop should not be the same variety to avoid the contamination due to volunteer plants.

Isolation requirement: For Seed production of tomato, varieties require minimum of 50m for foundation seed and 25m for certified seed. For hybrid seed production, it requires minimum of 200 M for foundation (parental line increase) and 100 M for certified hybrid seeds.

Seed rate: i) Varietal seed production– 300 to 400 g/ha. ii) F_1 hybrid seed production- Male parent@ 25 g/ha; Female parent@ 100 g/ha.

Seed Treatment: The seed required for one hectare are to be inoculated with Azosprillum. For this, the seeds should be first mixed with the required quantity of rice gruel and then with 150 g of Azosprillum after shade drying it can be used for sowing.

Nursery: Sow the seeds in raised nursery bed of 20 cm height, in rows of 5 cm gap and covered with sand. Eight and ten nursery beds will be sufficient to transplant in one acre.Apply 2 kg of DAP 10 days before pulling out of seedling.

Transplanting: Transplanting should be done with the seedlings are 20-25 days old, preferably at evening time.

Spacing: It varies with varieties from 60 x 30cm to 60 x 45 cm. In hybrid seed production, 90 x 60 cm for female parent and 60 x 45 cm for male parent.

Planting ratio: For hybrid seed production, the female and male parents are normally planted in the ratio of 12:1 or 12:2.

Manuring: After thorough preparation of a field to fine tilth, apply 25 tons of FYM per ha. Apply 100 : 100: 100 Kg of NPK/ha of which, 50% of the N is applied as basal dressing and remaining 50% of N as top dressing in two split doses at just before flowering and fruit formation stages.

Roguing: The roguing should be done based on the plant characters (determinate / indeterminate), leaf, branching and spreading characters and also based on fruit size, shape and colour. The plants affected by early blight, leaf spot and mosaic (TMV) diseases should be removed from the seed production field.

Specific field requirements

Factors	Foundation	Certified
Off types – variety (max)	0.1 %	0.2%
Hybrid (max)	0.01%	0.05%
Plants affected by seed borne diseases (max)	0.1 %	0.5%

Pest and disease management

Pests	Diseases
Fruit borer -*Helicoverpaarmigera* *Spodoptera litura* (common for both) Serpentine leaf miner Whitefly Nematode	Damping off (nursery) Leaf spot Leaf curl Tomato spotted wilt virus

Crossing technique for production of hybrids

In tomato the hybrid seed production is normally done by 'Emasculation and Hand Pollination'. Emasculation is done before the anthers are mature and the stigma has become receptive to minimize accidental self pollination. Thus emasculation is generally done in the evening, between 4 PM and 6 PM, one day before the anthers are expected to dehisce or mature and the stigma is likely to become fully receptive. The anther and surrounding cap are removed to prevent self-pollination. The petals and sepals are cut to identify the flower for future pollination. Emasculate the bud by hand with the help of needle and forceps. Remove the calyx, corolla and staminal column or anthers, leaving gynoecium *i.e.*, stigma and style intact in the flower. Emasculated flowers should be covered immediately with red coloured paper cover to protect against contamination from foreign pollen and also for easy identification of emasculated bud during dusting. Remove the red paper cover of the emasculated bud and dust the pollen gently over the stigmatic surface using cotton or camel brush, etc. After dusting, the emasculated flowers are again covered with white or other coloured paper cover for two to three days. Pollen collected from one male flower can be used for dusting 5 to 7 emasculated flowers. However use of chemical hybridizing agents (MH 1000 ppm) or CMS lines are also practiced.

Harvesting and seed extraction: The fruits are harvested after full maturity of the fruit when turn in to red color fruits from first and last one or two harvests should not be used for seed extraction. The fruits from in between 6 7 harvest should be used for seed extraction. The seed viability is depends on the method on which the seeds were extracted and hence, it is more important to choose proper methods of seed extraction. Before seed extraction, the fruits are to be graded for true to type and selection of medium to large size fruits for getting higher recovery of quality seeds.

The acid method of seed extraction is the best method for tomato seed extraction. In this method, the fruits are to be crushed into pulp and taken in a plastic containers (or) cement tank. And then add 30 ml of commercial Hydrochloric acid per kg of pulp, stir well and allow it for ½ hour. In between this duration the pulp may be stirred well for one or two times. This facilitates the separation of seed and pulp. After ½ hour, the seeds will settle down at the bottom and then the floating fraction is to be removed. The collected seeds should be washed with water for three or four times.

Table 1. Comparison of different seed extraction methods

Method	Fermentation	Acid	Alkali
	Mix fruit pulp with water - 24 - 48 h	HCl @10ml / Kg of pulp - 20-30 minutes	Washing soda @ 900mg/4 l of water- equal volume – overnight soak
Salient feature	Low cost. Unskilled labour. More time taken. Low seed recovery (0.5 to 0.6 %) Dull seed colour. Seed borne pathogens	Cost is more. Skilled labour. Lesser time. High seed recovery (0.8 to 1 %). Bright colour market value higher. Seed borne pathogen removed	Recovery 0.7 to 0.8 per cent. Luster of the seeds will be lost. Improper washing leads to injury to seeds

		Improper washing leads to injury to seeds	

While following acid method we must use only plastic or stainless steel containers or cement tank. Care must be taken to avoid the usage of iron or zinc containers, which will affect the viability potential of the seeds and as well, damage to the containers due to chemical reaction with acid.

For large scale seed extraction we can use the tomato seed extractor. The seeds extracted by this machine may again be treated with commercial Hydrochloric acid @ 2 3 ml/kg seed with equal volume of water for 3 5 minutes with constant stirring. And then seed should be washed with water for three to four times.

It is easy to dry the seeds extracted by acid method and also remove the fungus growth over the seed coat, thus seeds possess golden yellow colour and high vigour. The seed extracted by fermentation method posses poor vigour and off colour due to fungal activity.

After proper drying, the seed processing is essential. This will be helpful for maintaining high vigour and viability by way of removing immature and small seeds. In processing, we have to remove broken, immature and diseased seeds, other crop and weed seeds, mud and other inert matters. For processing tomato seeds, BSS 10 x 10 wire mesh sieve should be used.

Storage of unprocessed seeds results in poor viability. In processing, the sieves must be cleaned while changing to other variety otherwise it leads to physical admixture results in genetic contamination. Hence, utmost care must be taken during processing of seeds to maintain quality.

Seed treatment: The seeds may be treated with captan or Thiram @ 4g/kg of seeds. The seeds can also be treated with halogen mixture @ 5g/kg of seed and it is a ecofriendly seed treatment.

Storage containers: Seeds could able to absorb moisture from atmosphere. Hence for storing seeds in the coastal region (or) river sides we should use moisture vapour proof containers *i.e.*, 700 gauge polyethylene bags. For seed storage every time new containers must be used.

Seed Yield:100-120 Kg/ha.

Seed Standards (variety and hybrid)

Factors	Foundation	Certified
Pure seed (Min.)	98%	98%
Inert matter (Max.)	2%	2%
Other crop seeds (Maxi) No./Kg	5/kg	10/kg
Weed seeds (Max.)	None	None
Germination (Min.)	70%	70%
Moisture (Max.)	8%	8%
For VP container	6%	6%

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Quality Seed Production of Varieties/Hybrids in Okra

Dr.PidigamSaidaiah, Ph.D
Associate Professor, Dept. of Genetics and Plant Breeding, SKL Telangana State Horticultural University, College of Horticulture, Mojerla, Telangana

Climatic requirement: Okra is a warm season crop and thrive best during warm moist season although it grows fairly in hottest summer. However, it requires cooler night. It is susceptible to frost. The seeds of okra will not germinate below 16°C temperature, Optimum temperature for germination is 26-30°C. Under Haryana conditions seed yield of summer crop is much lower as compared to rainy season crop. Seed size of summer crop remains small. Moreover, early rains (May-June) at the time of pod maturity and drying impair the seed quality.

Flower characters and pollination: Bhendi is often cross-pollinated crop. Flower bud appears in the axil of each leaf from 4th to 8th leaf depending upon the cultivar. Flower bud takes about 22-26 days from initiation to full bloom. The time of anthesis varies with the cultivar, temperature and humidity and it ranges from 8 to 10 A.M. The dehiscence of anthers is transverse and occurs 15-20 minutes after anthesis. The pollen fertility is maximum during the period an hour before and an hour after opening of flower. The flowers remain open for a short time and they wither late in the afternoon. The stigma is receptive as flowers open. Under Indian conditions cross pollination by insects has been reported to the extent of 4.0 to 19%. Cross pollination to an extent of 12 per cent is due to protogynous. The extent of pollination at a particular place will depend upon the cultivar, competitive flora, insect population and season *etc.*

Methods of seed PRODUCTION: Breeder seed → Foundation seed → Certified seed

Varieties:CO.1, CO.2, MDU.1, Parbhani Kranti, Arka Anamika, Pusa A-4, PusaSavani, Punjab-7, JK Harita, Parbhawa

Season: June-July, September- October and February- March

Land requirement: Select field on which bhendi crop was not grown in the previous season, unless the crop was of the same variety and certified.Field should be free from wild bhendi (Abelmoschus sp.)

Isolation requirement: Seed field must be isolated from other varieties at least by 400m for foundation and hybrid seed production and 200m for certified seed production.

Seed rate: Varieties : 8-10 Kg/ha; Hybrids : 4 Kg/ha (female) : 1 Kg/Ha (Male)

Main field manuring: Apply 12.5 tons of FYM/ha before ploughing. Apply 150:75:75 kg NPK/ha, of which 50% of the N should be applied as top dressing in two split doses at flowering and 10 days later.

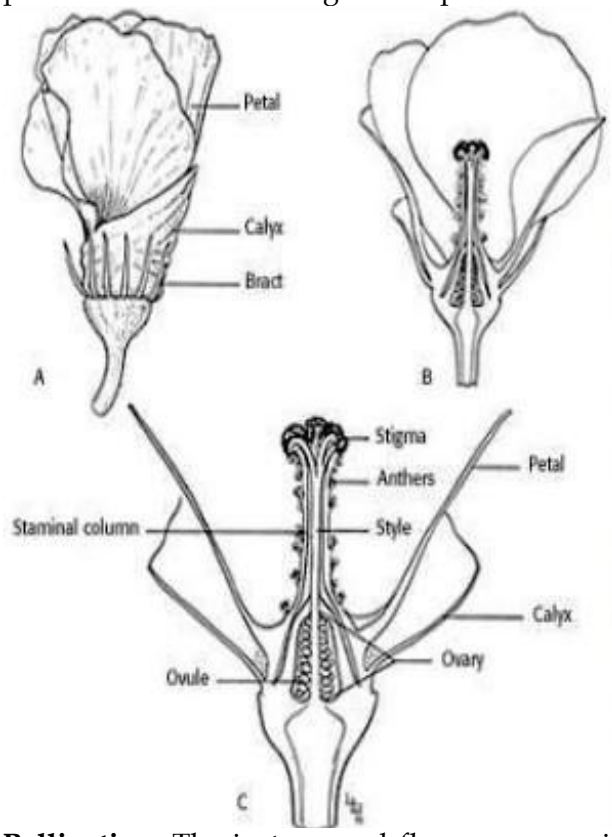
Planting ratio: For hybrid seed production, female and male parents are normally planted in the ratio of 4:1.

Roguing: Minimum of three inspections for varieties and 4 inspections for hybrids should be at 1. Vegetative, 2 & 3. Flowering and 4. Fruit maturity stages. The rouging should be based on the plant characters, hairyness, fruit character like fruit colour, number of ridges, fruit length *etc.*,

and the off type and mosaic attacked plants should be removed from the seed field. Wild bhendi if present should be removed before flowering.

Hybrid Seed Production of Okra:

The most productive and desirable hybrid seed obtained from the female parent when there is a perfect coincidence of stigma receptiveness and pollen viability.



Pollination: The just opened flowers were picked from the male parent in a separate brown paper pockets and used for crossing of emasculated flowers. Different colour thread was tied to the pedicel of the crossed buds for easy identification. Pollination was carried out daily between 8-00 am to 4-00 pm. The crossing was carried out for a period of eight weeks from the initiation of flowering and avoid the selfed seeds in the hybrid. Therefore, time of pollination and ratio between female to male flower crossing is to be optimized to get increased seed set and yield in female parent of bhendi.

Emasculation: The buds opened next day, were selected in female parent and emasculation was carried out by removing the androecium along with the corolla. These emasculated buds were covered with butter paper pockets to avoid cross pollination and also for easy identification of emasculated flower for pollination. The emasculation was carried out daily from 2-00 to 6-00 pm.

Distance between Male and Female fields: There should be a distance of 5 metres between male lines and female lines

Rouging: The rouging should be based on the plant characters, hairiness, fruit character like fruit colour, number of ridges, fruit length etc., and the off type and mosaic attacked plants

should be removed from the seed field. Wild bhendi if present should be removed before flowering.

Irrigation: Five to six protective irrigations are required for the whole crop period.

Field inspections: A minimum of three inspections shall be made, the first before flowering, the second during peak flowering and fruiting stage and the third at mature fruit stage and prior to harvesting.

Diseases and Pests

Insects	Diseases
• Shoot and fruit borer: (Eariassp) • Fruit Borer: *Helicoverpaarmigera* • Sucking Pests –Jassids: *Amrascabiguttulabiguttula* • Whiteflies:*Bemisiatabaci* • Green peach aphid: *Myzuspersicae* • Ants • Red Spider Mites • Root-knot nematodes	• Okra Vein Mosaic Virus (YVMV) Causative agent: Yellow Vein Mosaic Virus • Cercospora Leaf Spot causativeagent: *Cercosporaabelmoschi, C. Malayensis, C. hibisci* • Fusarium Wilt Causative agent: *Fusarium oxysporum f. sp. Vasinfectum* • Powdery Mildew Causative agent: *Erysiphe cichoracearum, Sphaerothecafuliginea* • Root-decaying disease. • EnationLeaf Curl of Bhendi • Damping Off Causative agent: Pythium spp., Rhizoctonia spp.

Specific field standards

Factors	Foundation	Certified
Off types (max)	0.1 %	0.2%
Objectionable weed (max)	None	None
Diseases affected plants(max)	0.1%	0.5%

Objectionable weed is wild Abelmoschus sp..Designated diseases– Yellow Vein Mosaic (Hibiscus virus-1)

<u>Hybrid seed production</u>

In bhendi, since the flowers are large in size, hand emasculation and pollination is the best suitable method for seed production. The emasculation and dusting can be done as per the methods outlined in tomato. The male and female parents are raised in blocks at the ratio of 9:1 (Female: Male).

Harvesting and threshing: Fruits should be harvested when they have dried (30-35 days after crossing). The pods which expose hairline crack and turned in to brown colour on drying alone are picked by hand and the seeds are separated manually. Then the seed are cleaned, dried and treated with Captan/ Thiram (2g / kg of seed).

It can be upgraded by water floatation technique (*i.e.*, the seed is immersed in a column of water (1:10 by volume). Stirred well and floaters (9-10%) containing insect damaged and ill-filled seeds are separated. The sinkers blackish olive green in colour that sinks down to the bottom are removed and dried.

In okra, the seed coat colour ranged from green to grey and finally to black. The discoloured seeds may not be acceptable as seed for sowing because of poor physical appearance and high expected incidence of seed borne fungi. The normal green colour seeds have better storage potential. Bhendi seeds dried to 7 per cent moisture, treated with captan (2g kg-1 of seed) and stored in a sealed 700 gauge polyethylene bag could maintain 80 percent germination up to 24months of storage.

Processing: Seeds are to be processed with BSS 7 wire mesh sieve.

Seed yield (OPVs): 10-15q/ha

Seed standards hybrid /varieties

Factors	Foundation Seed	Certified Seed
Pure seed (Min.)	99%	99%
Inert matter (Max.)	1%	1%
Other crop seeds (Max.) No./kg.	None	5/kg
Weed seeds (Max.) No./Kg.	None	None
Objectionable weed (Max.)	None	None
Other distinguishable varieties (ODV) (Max.)	10/kg	20/kg
Germination (Min.)	65%	65%
Moisture (Max.)	10%	10%
For VP container (Max.)	8%	8%

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Quality Seed Production of Varieties/Hybrids in Bitter Gourd

Dr. Pidigam Saidaiah, Ph.D
Associate Professor, Dept. of Genetics and Plant Breeding, SKL Telangana State Horticultural University, College of Horticulture, Mojerla, Telangana

Botany: Plants are monoecious annuals with medium size vines. Staminate flowers are small, yellow and borne on long slender pedicels. The pistillate flowers are solitary, have small pedicel and are easily distinguishable by oblong to long distinct green colour ovary.
Flowers are yellow. Leaves are segmented and have more length than width in the outline. There are five calyx and five corolla. Filaments are three, two are bilocular and one is unilocular. There are three short styles terminated by three bilobed or divided stigma. Anthesis and dehiscence occur early in the morning. Therefore, selfing and crossing should be attempted in forenoon preferably in early hours. Anthesis time: 5.00- 10.30 AM; Anther dehiscence occurs between 7.00-8.00 AM and Stigma receptivity ranges between 1 day before anthesis and 1 day after anthesis.

Varieties: CO 1, MDU 1, PusaVisesh, Pusa Do Mausami, VK1 Priya, ArkaHarit, Konkan, Preethi

Hybrids: CBGH 1, Pusa Hybrid 1

Season: June-July and January – February.

Sowing: Sowing pre-germinated seeds to maintain optimum field population, the seeds are soaked in water for 24 hours. Then place the seeds in moistened sand and cover the seeds with sand and left for 3 days. Maintain the sand in wet condition. After 3 days the seeds with protruding 60ouging are separated and used for sowing.

Seed rate: 1.8 kg /ha

Spacing: 45 x 45 x 45 cm at 2.5 x 2 m, sowing of three seeds per pit at 2cm depth micropylar end facing upward is favorable for better field emergence.

Manuring: Application of 10 kg FYM per pit

Top dressing: Urea 22 g / pit during 1st flowering. Urea + potash 18 + 5 gm/pit at 20 days after flowering. Urea 18 gm + potash 5 gm/ pit at 40 days after flowering.

Foliar application: Spraying ethrel@ 200 ppm from 4 leaves stage onwards at one week interval for four times.

Weeding: Field has to be maintained clean by frequent hand weeding in order to remove objectionable weed species like *Mimordicabalsamina*. L., *M. cochinchinens* and *M. diocia* ex wild.

Main field maintenance: Each plant has to be provided with stacking 20-25 days after sowing for training the vines over the pandal. Training operation has to be carried out daily until the fruiting stage.

Irrigation: Irrigated the crop before dibbling the seeds and thereafter once in a week.

Roguing: During vegetative, fruit formation and fruit harvest phases, 60ouging is attempted based on plant characters like height of plant, leaf shape, size, surface of leaf and fruit characteristics like length of fruit, size, shape and colour.

Plant protection

Pests: Beetles, fruit flies and caterpillars: Spray malathion 50 EC 1ml/lit or dimethoate 30 EC 1ml/lit or methyl demeton 25 EC 1ml/lit or fenthion 1000 EC 1ml/lit. Avoid copper and sulphur dust since these chemicals cause injury to the plants.

Diseases:

Powdery mildew- control: Spray Dinocap 1ml/lit or Carbendazim 0.5 g/lit.

Downy mildew- control: Spray Mancozeb or Chlorothalonil 2 g/lit twice at 10 days interval.

Field Standards

Contaminants	**Minimum distance (meters)**	
	Foundation	**Certified**
Fields of other varieties	1000	500
Fields of the same variety not confirming to varietal purity requirements for certification and from balsam apple : Momordica balsamina L.; M. cochinchinensisSpreng; .dioica Roxb.	1000	500

Specific requirements

Factor	**Maximum permitted %**	
	Foundation	Certified
*Offtypes	0.10	0.20
**Objectionable weed plants	None	None

* Maximum permitted at and after flowering.

** Objectionable weeds shall be: *Momordica balsamina* L.; *M. cochinchinensis*Spreng; *.dioica*Roxb.

Seed Standards

Factor	**Standards for each class**	
	Foundation	**Certified**
Pure seed (minimum)	98.0%	98.0%
Inert mater (maximum)	2.0%	2.0%
Other crop seeds (maximum)	None	None
Total weed seeds (maximum)	None	None
Objectionable weed seeds	None	None
Other distinguishable varieties (maximum)	5/kg	10/kg
Germination (minimum)	60%	60%
Moisture (maximum)	7.0%	7.0%
For vapour proof containers (Maximum)	6.0%	6.0%

Harvest: The harvest starts from 60-65 days after sowing and matured fruits can be harvested once in a week. The fruits with yellow or yellowish orange colour should be harvested for seed extraction.

Seed yield:60-120 kg/ha

Method of hybrid seed production: Isolation distance for breeder/foundation and certified seed is 800 m and 400 m respectively. 1000 seed weight is 175 g and seed yield are about 6 q/ha.

For hybrid seed production in India, 150 g female parent seed and 40 g male parent seed are planted in separate blocks within 1 unit of land (1000 m^2).

The female and male flower buds are covered and U clipped with small paper bags in afternoon, prior to pollination next morning. Next morning open male flowers from male parent are collected and used in pollination of already covered female flowers which have opened by now. Pollinated flowers are again covered and a thread is tied around pedicel to identify the crossed fruits. Open-pollinated fruits are removed as and when noticed.

Under insect proof net; covering of female and male flowers could be skipped. Pollination can be performed daily in morning hours using open/male flowers as source of pollen grain on to the stigma of freshly opened female flowers. The crossed flowers need to be identified by tying a thread around pedicel. The seed multiplication ratio under normal situation is about 100.

Removal of staminate flowers+ hand pollination or Gynocecism and natural pollination is in general followed to produce hybrids in bitter gourd.

Seed extraction: Fruits are cut longitudinally and the seeds are removed along with mucilaginous material and then the seeds are washed with water.

Drying: The seeds are dried under shade for one or 2 days followed by drying under sun to reduce the moisture content 7-8% for storing in cloth bag and 6% for moisture vapour proof containers.

Grading: For grading the seeds BSS 4 x 4 size sieves are used

Storage: Seed treating chemicals captan or thiram 4 gm /kg of seeds or halogen mixture @ 5 g/kg of seeds is recommended.

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Quality seed production in Cauliflower: Varieties and hybrids

Dr Kartikeya Srivastava
Department of Genetics & Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi

Cauliflower is an important vegetable crop of the family Brassicaceae and is grown in many countries of the world. India ranks second after China in cauliflower production. India produced 8.94 million tones of Cauliflower in the year 2019-20 from 0.46 million ha area with an average productivity of about 18.3 mt/ha. Important cauliflower growing states are Uttar Pradesh, Karnataka, West Bengal, Punjab Bihar and Himachal Pradesh.
Cauliflower requires a cool moist climate for seed production. The optimum monthly average temperature is 15-20 degree centigrade. Early varieties require relatively higher temperature and longer day-length than late varieties.

Land to be used for seed production should be free from volunteer plants. The soil should be deep, fertile, well supplied with organic matter having pH value 5.5-6.

Method of seed production

- There are two methods of seed production

1. In situ method (seed to seed method)
2. Transplanting method (Head to seed method)

- For seed production, seed to seed method is recommended since the head to seed method in India has not been very successful.
- In seed to seed method (In situ method) the crop is allowed to over winter and produce seed in the original position, where they are first planted in the seedling stage.

Stages of seed production
Breeder seed→ Foundation seed→Certified seed

Varieties
Early
Kashi Kunwari, Early Kunwari, Pusa Katki, Early Patna, Pusa Deepali, Pusa Early Synthetic, Pant Gobhi3, Improved Japanese,

Mid season
Pant Shubhra, Pusa Synthetic, Pusa Shubhra, Pusa Aghani, Selection 235S, Hisar No.1, Pusa Himjyoti, Kashi Agahani

Late
Kashi Gobhi-25, Snowball 16, Pusa Snow ball 1, Pusa Snowball 2, PSK 1, Pusa hybrid -2

Season
In the hills, the last week of August is the optimum sowing time.

- The seed is sown in a nursery and transplanting should be completed by the end of September.
- For early varieties (in plains) best season for sowing is the last week of May and transplanting should be completed during first week of July.

- In hills, sowing should be adjusted that the plants put up the maximum leafy growth by 15th December when the temperature goes down and plants become dormant for which last week of August is optimum and transplanting should be completed by the end of September.
- The mean temperature of 6.5 to 11° C during February to March is very conducive to curd formation.

Land requirement

- In the hills, select field on which the same kind of crop or any other cole crop was not grown in the pervious two years, unless the crop within the previous two years, was field inspected by the certification agency and found not to contain seed born diseases infection beyond the maximum permissible limit.

Isolation requirement

- Cauliflower is mainly a cross pollinated crop. Pollination is chiefly done by bees. The seed field must be separated from fields of other varieties at least by 1600 m for foundation class and 1000 m for certified class seed production.

Seed rate

- 375 to 400 g /ha.

Nursery

- Seeds may be sown on raised nursery beds 15 20 cm height in rows with 10 cm spacing.
- Twenty five nursery beds of 2 to 2.65 m x 1 to 1.25 m size are enough for one hectare.
- Thin sowing should be done to avoid damping off.
- Three tonnes of FYM should be applied to nursery bed.
- DAP spray at 10 to 15 days after germination is important.
- Apply lime @ 5 t/ha before one month to nursery field and apply Borax and Sodium molybdate @ 4 kg/ha before sowing.

Transplanting

- Transplant the seedlings at 35 40 days old preferably at evening time with the spacing of 60 x 45 cm (for early varieties in plains) or 90 x 60 cm for late variety and irrigate immediately after transplanting.

Main field manuring

- The field should be prepared to fine tilth by deep ploughing and three to four harrowing followed by levelling. Cauliflower crop requires heavy manuring. Apply 50 60 tons of FYM/ha at the time of land preparation.

Foliar application

- NAA @ 40 ppm sprayed at 30 days after curd initiation was superior in increasing the yield and quality of seed (Gurusamy, 1996).

Roguing

- Minimum of four inspections are required viz., pre marketable stage, initiation of curd stage, curd formed stage and flowering stage. Roguing should be done based on the curd size, shape and colour, when fully developed. Off type plants with poor curd formation and plants affect by designated diseases like black leg, black rot, soft rot, leaf spot and phyllody should be removed during roguing.

- First roguing is done after curd formation. Plants forming loose ricey, fuzzy and buttons are rejected. Blind, deformed and diseased plants are also rejected. Second roguing is done after bolting but before flowering, plants with peripheral and uniform bolting are kept for seed production. Early and late bolters are also rejected.

Field standard

Contaminants	Maximum permitted (%)	
	Foundation	Certified
Off types *	0.10	0.20
Plants affected by seed borne diseases **	0.10	0.50
Plants affected by phyllody **	0.1	0.5

Pest and disease management

- Use of insecticides during flowering affects the insect pollinators and will result in poor seed set. A single soil application of granulated Phorate, Dimethoate @ 18 Kg/ha during early February for control of sucking pests (Aphids) is advisable. In cauliflower the major disease in "Damping – off". Thin showing and drenching with 150 g of Bavistin in 100 liters of water will control the disease.

Plant protection

Pests

Cut Worms

Set up of light trap in summer months. Spray chlorpyriphos 2 ml/lit in the collar region during evening hours.

Aphids

The incidence is severe during autumn season. Installation of yellow sticky trap at 12 no/ha to monitor Macropterous adults (winged adult) is necessary. Spraying of neem oil 3 % or dimethoate 2 ml/lit with 0.5 ml Teepol/lit.

Diamond backmoth

1. Growing mustard as intercrop at 20:1 ratio.

2. Installation of pheromone traps at 12 No/ha.

3. Spraying of cartap hydrochloride 1 g/lit or Bacillus thuringiensis 1g/lit at primordial stage (ETL 2 larvae/plant)

4. Spraying of NSKE 5 % after primordial stage.

5. Release of larval parasite Diadegma semiclausum (Ichneumonidae: Hymenoptera) at 50,000/ha, 60 days after planting.

Diseases
Club root

- Seed treatment at 10 g/ kg of seeds or soil application @ 2.5 kg/ha or seedling dip in solution of 5g/ litre with Pseudomonas fluorescens.
- Dipping the seedlings in Carbendazim solution (1 – 2 g/lit) for two minutes.
- Drench the soil around the seedlings in the main field with Carbendazim @ 1 g/lit. Follow crop rotation.
- Crucifers should be avoided for three years.

Leaf Spot
Spraying of Mancozeb at 2 g/lit or Carbendazim 1 g/lit.

Leaf Blight
Spray of Mancozeb @ 2.5 g/ litre.

Blanching
Blanching refers to covering of curds. A perfect curd of flower is pure white. It is necessary to exclude sunlight to obtain this. The common practice is to bring the outer leaves up over the curd and tie them with a twine or rubber band. By using a different coloured twine each day. It is easy at the time of harvest to select those tied earlier.

Physiological disorders
Browning or brown rot
This is caused by Boron deficiency. It appears as water soaked areas and later changes into rusty brown. Spray one kg of Borax in 500 lit of water 30 days after planting.

Whip tail
This results from the deficiency of Molybdenum. It is more pronounced in acidic soil. The leaf blades do not develop properly. In severe cases only the midrib develops and it can be corrected by spraying 100 g of Sodium molybdate in 500 lit of water 30 days after planting.

Buttoning
The term buttoning is applied to the development of small curds or buttons. The plants do not develop normally and leaves remain small and do not cover the developing curds. Deficiency of Nitrogen and planting the early varieties in late season may cause these symptoms. Avoid transplanting aged seedlings.

Blindness
Blind-cauliflower plants are those without terminal buds. The leaves are large, thick, leathery and dark green. It is due to the prevalence of low temperature when the plants are young or due to damage to the terminal bud during handling the plants or due to injury by pests

Scooping
Scooping central portion of curd when it is fully formed helps in the early emergence of flower stalks in hills. Scooping is normally not required for seed production in plains. Scooping curd pruning and half curd removal were effective in increasing the seed yield. However, scooping of curd was best compared to other methods

Harvesting &processing
The ripened fruit is called siliqua. Harvesting may be done in two lots. Heavy bearing may topple the plants, hence staking may be done wherever necessary. Wind belts can also be

erected if needed. Generally the early matured plants are harvested first, when the siliqua turn in to brown colour. Delayed harvest results into seed shattering and bird damage. Hence, 2 3 harvestings are required. About 50 days are needed for pod maturity after fertilization. Seeds of early types are ready for harvesting in December January and in February March for North Indian Plains. However, snowball types are ready for harvesting by June. As harvesting is done when bottom siliqua turn brown followed by yellowing of the top siliqua, curing is necessary for ripening the late maturing siliqua. After harvesting, plants are piled up for curing. After 4 to 5 days it is turned up side down and for further curing for 4 to 5 days. The siliqua are threshed with pliable sticks and cleaned. Then the seeds are dried to 7% moisture content, cleaned and treated with Bavistin @ 2 g / Kg of seed.

Seed yield

Indian cauliflower may very between 500 600 kg/ha and snowball from 300- 500 kg/ha.

Designated diseases

Black leg, Black rot and Soft rot

Hybrid seed production in cauliflower using self-incompatibility

In recent years, a number of hybrid varieties have been developed in cauliflower by public and private concerns. In cauliflower, F1 hybrids have been found advantageous for earliness, high early and total yield, better curd quality with respect to compactness and color, uniform maturity, resistance to insects, pest, diseases and unfavorable weather conditions. In India, F1 hybrids in cauliflower currently share only 3% of the total seed requirement for cauliflower, and the rest, 97% of the seeds for the crop, are open pollinated. However, the acreage under F1 hybrids is increasing every year. Therefore, there is a tremendous potential for the development of hybrids in cauliflower. The main hindrance to the popularization of F1 hybrids is non-availability and high cost of hybrid seed. Due to these reasons poor farmers can not grow it. Whatever, hybrid seeds are popularized are developed by private sector. In spite of several advantages of hybrids to the farmers, the public sector hybrids are very few such as Pusa Hybrid-2, Pusa snowball hybrid-1 and Pusa Kartik Sankar. Scientists have good scope to develop region specific cauliflower hybrids and to educate farmers for hybrid seed production of cauliflower. This can ensure the hybrid seed production in farmer's participatory mode. Seed village concept can also work well. In India self-incompatible lines in Indian cauliflower have been developed to be used in hybrid seed production. In relation to pollination, cauliflower genotypes are generally cross pollinated, but self-pollination could occur especially for the summer types. Bees, blowflies and bumblebees often are responsible of the cross-pollination.

The strength of incompatibility can be scored by comparing the number of seed set after each specific self- or cross-pollination at specific location of flower/ flower buds, 60 days after pollination. Seed counts at maturity stage often do not reflect degree of expression. In this method a string is tied at the mid point of inflorescence below which the flower buds have just started flowering and above which unopened buds are located. Scores for strength of self incompatibility counting the number of seed set in self pollinated capsules above and below the ring. If no seed set is there in capsule above the ring (unopened flower bud) it is considered highly self incompatible, since the incompatibility reaction has began at bud stage only. Such lines can be taken in hybrid seed production in isolation since self incompatibility will yield true hybrid.

However, self in compatibility can also be studied by dissection method of stigma and staining by dyes. The fluorescent microscopic observations are taken on pollen ability to penetrate style (within 12-15 h).

Keeping self incompatibility intact the cauliflower inbred lines are selected for yield, quality, maturity and horticultural traits which are as follows:

- Numbers of outer leaves
- Colour of curd & outer leaves
- Vigour
- Maturity (extra early/early/mid/late)
- Curd shape
- Curd firmness/ density
- Curd net weight
- Stem length
- Blanch
- Resistance to biotic & abiotic stresses

Development of homozygous SI inbreds is done by forced selfing of self-incompatible plants in following methods:

- Bud pollination (Widely used)
- Treatment of flowering cauliflower plants with CO2 gas under enclosed chamber by putting dry ice in it (CO2 enrichment)
- Common salt (NaCl) 5% solution spray at flowering coupled with honey bee pollination
- Tissue Culture: for mass multiplication of stable inbred lines.

Two strong self incompatible but cross compatible selected inbred lines with good combining ability and high heterosis and desirable horticultural traits are grown in alternate rows isolation with recommended spacing of 60X45 cm. Spacing can be more for late groups. If synchronized flowering between male and female rows are not there then nicking (staggered dates of transplanting) is done to achieve synchronized flowering. At the time of curd marketable stage off type/ unhealthy plants are removed from plot.3- 4 honeybee boxes per acre are placed in the seed production plots. Non-bolting, tight buttons of cauliflowers are removed to facilitate the bolting and flowering. Staking of cauliflower plants with sticks are done to avoid lodging. At the time of flowering spray of such insecticide which repel honeybee should be avoided. Harvesting of matured and dried capsules should be done if both parents are SI then hybrid seeds can be obtained from all the rows. If only one parent is SI (female) then after completion of flowering non self incompatible (male) rows should be removed to avoid mixing, and hybrid seeds are harvested from SI (female) rows at the time of maturity. Capsules are threshed; seeds are dried in shade to moisture level 10% and stored in cotton bags.

Requirement for cauliflower hybrid seed production:

- Strong SI lines or stable male sterile lines with good combining ability and high heterosis.
- Isolation distance from other Cole crops
- Rouging of off types to maintain purity of hybrid seeds
- Pollinators – Honeybee boxes (3-4 boxes per acre)
- Stalking of the plants at the time of flowering to prevent lodging
- Synchrony in flowering between male and female lines

Maintenance of genetic purity in vegetable crops

Kartikeya Srivastava, Department of Genetics & Plant Breeding, Institute of Agricultural Sciences, B.H.U.Varanasi-221005, email: karstav@yahoo.com

If there is any deterioration in the genetic makes up of the variety during seed multiplication and distribution cycle, there would definitely be proportionate decrease in its performance e.g. yield, disease resistance etc. Quality seeds has to meet the minimum seed certification standards and quality attributes viz., physical purity, germination per cent, moisture content, seed health and genetic purity. The genuineness of the variety is one of the most important characteristics of good quality seed. Genetic purity test is done to verify any deviation from genuineness of the vegetable variety during multiplication stages. For certification genetic purity test is compulsory for all foundation and certified hybrid vegetable seeds. Higher genetic purity is an essential requirement for the commercialization of vegetable seed. Genetic purity refers to the percentage of contamination by seeds or genetic material of other varieties or species. In other words, genetic purity of seeds refers to the trueness to type. If the seed possesses all the genetic qualities that breeder has placed in the vegetable variety, it is said to be genetically pure. In general, the genetic purity of the seed planted must equal or exceed the final product purity standard required, as purity generally decreases with each subsequent generation of propagation.

It is virtually impossible to assure that no off-type plants or pollen is present in the vegetable seed production field and that all handling and conveyance equipment and storage facilities are completely free of contamination. As a result, commercial planting seed is seldom 100% pure. In practice, practical seed genetic purity standards have been established by state seed laws and by seed certification agencies to ensure that the purchaser receives seed that is within certain purity tolerances.

These tolerances are established based on the biology of the species (i.e., self- or cross-pollinated), the type of variety (i.e., open-pollinated, hybrid, synthetic), and market-driven standards for final product quality. Earlier generations of seed (e.g., foundation or registered seed) have stricter standards in order to be able to meet the certified seed purity criteria.

Causes for Deterioration of Genetic Purity

The genetic purity of a variety or trueness to its type deteriorates due to several factors during the production cycles.

Kadam (1942) listed the following important factors responsible for deterioration of varieties.

1. Developmental Variation
2. Mechanical Mixtures
3. Mutations
4. Natural Crossing
5. Genetic drift
6. Minor Genetic Variation
7. Selective influence of Diseases
8. Techniques of the Breeder
9. Breakdown of male sterility
10. Improper / defective seed certification System

1. **Developmental Variation:** When a seed crop is grown in difficult environmental conditions such as different soil and fertility conditions, under saline or alkaline conditions or under different photo-periods or different elevations or different stress conditions for several consecutive generations the developmental variations may arise as differential growth response.

To avoid or minimize such developmental variations the variety should always be grown in adaptable area or in the area for which it has been released. If due to some reasons (for lack of isolation or to avoid soil born diseases) it is grown in non-adaptable areas it should be restricted to one or two seasons and the basic seed i.e. nucleus and breeder seed should be multiplied in adaptable areas.

2. **Mechanical Mixtures:** This is the major source of contamination of the variety during seed production. Mechanical mixtures may take place right from sowing to harvesting and processing in different ways such as;

a. Contamination through field – self sown seed or volunteer plants
b. Seed drill – if same seed drill is used for sowing 2 or 3 varieties
c. Carrying 2 different varieties adjacent to each other.
d. Growing 2 different varieties adjacent to each other.
e. Threshing floor
f. Combine or threshers
g. Bags or seed bins
h. During seed processing

To avoid this sort of mechanical contamination it would be necessary to rogue the seed fields at different stages of crop growth and to take utmost care during seed production, harvesting, threshing, processing etc.

3. **Mutations:** It is not of much importance as the occurrence of spontaneous mutations is very low i.e. 10-7. If any visible mutations are observed they should be removed by rouging. In case of vegetatively propagated crops periodic increase of true to type stock would eliminate the mutants.

4. **Natural Crossing:** It is an important source of contamination in sexually propagated crops due to introgression of genes from unrelated stocks/genotypes. The extent of contamination depends upon the amount of natural cross-fertilization, which is due to natural crossing with undesirable types, offtypes, and diseased plants.

On the other hand natural crossing is main source of contamination in cross-fertilized or often cross-fertilized crops. The extent of genetic contamination in seed fields is due to natural crossing depends on breeding system of the species, isolation distance, varietal mass and pollinating agent. To overcome the problem of natural crossing isolation distance has to be maintained.

Increase in isolation distance decreases the extent of contamination. The extent of contamination depends on the direction of the wind flow, number of insects presents and their activity.

5. **Genetic drift:** When seed is multiplied in large areas only small quantities of seed is taken and preserved for the next years sowing. Because of such sub-sampling all the genotypes will not be represented in the next generation and leads to change in genetic composition. This is called as genetic drift.

6. **Minor Genetic variation:** It is not of much importance; however some minor genetic changes may occur during production cycles due to difference in environment. Due to these changes the yields may be affected. To avoid such minor genetic variations periodic testing of the varieties must be done from breeder's seed and nucleus seed in self-pollinated crops minor

genetic variation is a common feature in often cross-pollinated species; therefore care should be taken during maintenance of nucleus and breeder seed.

7. **Selective influence of Disease:** Proper plant protection measures much be taken against major pests and diseases otherwise the plant as well as the seeds get infected.

a. In case of foliar diseases the size of the seed gets affected due to poor supply of carbohydrates from infec ted photosynthetic tissue.

b. In case of seed and soil borne diseases like charcoal rot of bean, downy mildew and *Sclerotinia* curd rot of cauliflower and wilt of pea, it is dangerous to use seeds for commercial purpose once the crop gets infected.

c. New crop varieties may often become susceptible to new races of diseases are out of seed production programms. Eg. Surekha and Phalguna became susceptible to gall midge biotype 3.

8. **Techniques of the Breeder:** Instability may occur in a variety due to genetic irregularities if it is not properly assessed at the time of release. Premature release of a variety, which has been breed for particular disease, leads to the production of resistant and susceptible plants which may be an important cause of deterioration. When popular vegetable varieties were released in India for commercial cultivation the genetic variability in both the varieties was still in flowering stage and several secondary selections were made by the breeders.

9. **Breakdown of male sterility:** Generally in hybrid seed production if there is any breakdown of male sterility in may lead to a mixture of F1 hybrids and selfers.

10. **Improper Seed Certification:** It is not a factor that deteriorates the crops varieties, but is there is any lacuna in any of the above factors and if it has not been checked it may lead to deterioration of crop varieties.

Maintenance of Genetic Purity during seed Production

Horne (1953) had suggested the following methods for maintenance of genetic purity;

1. Use of approved seed in seed multiplication of vegetable.
2. Inspection of seed fields of vegetable prior to planting
3. Field inspection and approval of the Crop at critical stages for verification of genetic purity, detection of mixtures, weeds and seed borne diseases.
4. Sampling and sealing of cleaned lots
5. Growing of samples with authentic stocks or Grow -out test

Various steps suggested by Hartman and Kestar (1968) for maintaining genetic purity are as follows;

1. Providing isolation to prevent cross fertilization or mechanical mixtures
2. Rouging of vegetable seed fields prior to planting
3. Periodic testing of varieties for genetic purity
4. Grow in adapted areas only to avoid genetic shifts in the variety
5. Certification of seed crops to maintain genetic purity and quality
6. Adopting generation system33

Safe guards for maintenance of genetic purity

The important safe guards for maintaining genetic purity during seed production are;

1. Control of seed source
2. Preceding crop requirement
3. Isolation
4. Rouging of seed fields
5. Seed certification
6. Grow out test

1. **Control of Seed Source**: The seed used should be of appropriate class from the approved source for raising a vegetable seed crop. There are four classes of seed from breeder seed, which are given and defined by Association of Official Seed Certification agency (AOSCA).

a. **Nucleus Seed:** It is handful of seed maintained by concerned breeder for further multiplication. The nucleus seed will have all the characters that he breeder has placed in it and it is of highest genetic purity. The quantity of nucleus seed is in kilograms.

b. **Breeder Seed:** It is produced by the concerned breeder or sponsoring institute or and which is used for producing foundation seed. It is of 100% genetic purity. The label/tag issued for B/s is golden yellow in color. The quality of breeder seed is assured by the monitoring team constituted by the govt.

c. **Foundation Seed:** It is produced from breeder seed and maintained with specific genetic identity and purity. It is produced on govt. farms or by private seed producers. The quality of foundation seed is certified by certification agency. It has genetic purity of above 98%. The certification tag or label issued for F/s is white in color.

2. **Preceding Crop requirement:** This has been fixed to avoid contamination through volunteer plants and also the soil borne diseases.

3. **Isolation:** Isolation is required to avoid natural crossing with other undesirable types, off types in the fields and mechanical mixtures at the time of sowing, threshing, processing 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 of seed.

4. **Rouging of Seed Fields:** The existence of off type plants is another source of genetic contamination. Off type plants differing in their characteristics from that of the seed crop are called as off types. Removal of off types is referred to as roughing.

The main sources of off types are

a. Segregation of plants for certain characters or mutations

b. Volunteer plants from previous crops or

c. Accidentally planted seeds of other variety

d. Diseased plants

Off type plants should be rouged out from the seed plots before they shed pollen and pollination occurs. To accomplish this regular supervision of trained personnel is required.

5. **Seed Certification:** Genetic purity in seed productions maintained through a system of seed certification. The main objective of seed certification is to make available seeds of good quality to farmers. To achieve this qualified and trained personnel from SCA carry out field inspections at appropriate stages of crop growth. They also make seed inspection by drawing samples from seed lots after processing. The SCA verifies for both filed and seed standards and the seed lot must confirm to

get approval as certified seed.

6. **Grow-out Test :** varieties that are grown for seed production should be periodically tested for genetic purity by conducting GOT to make sure that they are being maintained in true form. GOT test is compulsory for hybrids produced by manual emasculation and pollination and for testing the purity of parental lines used in hybrid seed production.

Marker based detection of purity of hybrid seeds (Hybridity)

It is estimated that for every 1% impurity in the hybrid seed the yield reduction is 100 kg per hectare (Mao *et al*., 1996). Maintenance of parental line purity is a prerequisite to ensure high genetic purity of hybrid seeds. A set of qualitative and quantitative characters known as descriptors are currently in use for variety identification and description. Some of these characters, particularly those showing quantitative inheritance, interact with the environment in which the variety is grown and thus make the process of variety identification subjective.

Marker is a 'TAG' for identification. It may be morphological or biochemical or molecular markers. Ideal marker should be polymorphic, Co-dominant, distributed evenly through out genome, heritable, independent to environmental factors and stage of the crop, easy, quick and cost effective to conduct and results must be reproducible

Genetic purity of a given seed lot can be assessed by using one of the following methods

1. Conventional grow out test
2. Chemical test
3. Electrophoresis method
    a. Biochemical markers (Proteins and Isozymes)
    b. Molecular markers (DNA)

Molecular markers, in contrast, being based on DNA sequence variation, provide an unbiased means of identifying crop varieties. The Biochemical and Molecular Techniques group of the International Union for the Protection of New Varieties of Plants (UPOV) is evaluating different DNA marker parameters prior to its routine use in establishing distinctness, uniformity and stability (DUS) of plant varieties.

This is a new approach to test the genetic purity of the seeds done at nucleotide level. This test screen through whole genome and produces enough polymorphism in closely related parental lines also. DNA markers have advantages over morphological and biochemical markers. Those are the resultant on genotype and environmental effect. While, DNA markers are resultant of only genotype of the cultivar and are independent to environmental conditions. The markers are dominant and Co-dominant and can differentiate between homozygous and heterozygous conditions of the cultivar. This is rapid to conduct and results are reproducible.

The banding pattern appeared at a particular molecular weight is used for the identification of parental lines, hybrid and off-types.

AA x aa

DNA band A1 DNA band A2

Aa

DNA band A1A2 with co-dominant markers

Identifying breeding lines and determining hybrid purity are major requirements in plant breeding and seed production. To test the conformity of hybrid seed, one must be able to distinguish the true hybrid resulting from cross between the male and female parents and one coming from self pollination of the female parent. The DNA finger printing of parental lines, hybrid and offtypes are used as a data base to identify off-types in the seed lot under question.

Molecular markers which could clearly distinguish the hybrids form its parental lines have been identified and developed in major agriculture and horticulture crops are RFLP, RAPD, SSR and AFLP.

Steps involved in molecular marker: Steps involved in molecular marker based genetic purity test are sophisticated but simple, environment independent and quick. These steps are:

a) Extraction of DNA from seed or leaves of young seedlings by CTAB method.
b) Polymerization of DNA with specific primers (PCR reactions).
c) Gel-electrophorasis of the PCR product.

d) Gel documentation of DNA bands
e) Analysis of results obtained in terms of purity or impurity of hybrids/genotypes.

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Principals and practices of quality seed production of vegetable crops

Nakul Gupta, Shivam Kumar Rai, ChendraSekher and P.M Singh
ICAR-Indian Institute of Vegetable Research, Varanasi 221305, U.P.

Introduction

Increasing health consciousness among peoples and to meet the nutritional security of country, consumption of vegetables has increased tremendously in recent years. To meet this vegetable demand quality seedwill playa pivotal role, since vegetable productivity is directly related to the genetic potential of the seed planted. It is estimated that the direct contribution of quality seed alone to the total production is about 15–20% depending upon the crop and it can be further raised up to 45% with efficient management of other inputs. Seed production is a biological process, which involves multiplying small quantities of nucleus/breeder/parental lines seed into larger quantities (certified/truthful labelled seed/hybrids) for commercial distribution, following specified stages over successive cropping seasons. Since seed is living, it is subject to the natural phenomena of aging and death. Besides, the growth of plant and the quality of seed production are strongly influenced not only by genetic factors but also by the environmental condition. Therefore, careful handling and monitoring is essential starting from field preparation to seed harvesting or during transportation and storage. Although, the package and practices of seed production technology vary from location to location and from crop to crop. But a general recommendation can be adopted for vegetable seed production. Ideally, quality seed should have following characters: 1. It should be true to its type (genetically pure). 2. It should be free from ad mixture of other variety of seeds. 3. It should have high percentage of germination. 4. It should be free from seed borne diseases.

Classes of Seed

1. *Nucleus Seed*: It is produced by the Breeder and it is genetically pure seed. 2. *Breeder Seed*:It is produced by the breeder from Nucleus Seed. Golden yellow colour tag is affixed by the breeder. 3. *Foundation Seed*: It is produced by the breeder seed under the supervision of the concerned seed Certification Agency. White colour tag certified by the certification agency is affixed. 4. *Certified Seed*: It is produced from the foundation seed. Certified seed may be the progeny of certified seed provided this reproduction does not exceed three generation beyond foundation seed stage I. It is determined by the seed certification agency. Certification tag shall be of azure blue colour for certified seed class.

Table-1 Genetic purity standard for different class of seeds and hybrids

Class of seed	**Genetic purity required**
Breeder seed	100
Foundation seed	99.5
Certified seed	99
Certified hybrid seed	95
Certified hybrid seed developed by Hand emasculation	90

General principles of seed production

Extreme attention is needed to the maintenance of genetic purity and other qualities of seeds at the time of hybrid seed productionin order to exploit the full yield potential ofdeveloped hybrid. In other words, hybrid seed production must be carried out under standardized and well-organized manner.Basically, there are two types of seed production principles.
(A) Genetic principles (B) Agronomic principles.

(A) Genetic Principle – It involves all the factors which may lead deterioration of genetic purity (true to type) of a crop variety. In negligence of genetic principles during seed production programme leads deterioration of the varieties. The important factor forvarietaldeterioration are listed by Kadam (1942):
(1) Developmental variation: When the seed crops are grown in difficult environment, under different soil and fertility conditions, or different climate conditions, or under different photoperiods, or at different elevation for several consecutive generations. The developmental variation may arise sometimes as differential growth response. To minimize the opportunity for such shifts to occur in varieties it is advisable to grow them in their areas of adaptation and growing seasons.
(2) Mechanical mixtures: Mechanical mixtures may often take place at the time of sowing, harvesting, processing, grading and packaging. If more than one variety is sown with same seed drill or volunteer plants of the same crop present in the seed field or different varieties grown in adjacent fields may cause mechanical mixing. Often the seed produce of all the varieties are kept on same threshing floor, grading is done with same grader and packaging is done in the old gunny bags etc. these practices may also cause mechanical mixing. To avoid mechanical contamination, it would be necessary to rogue the seed fields timely and practice the utmost care during the seed production, harvesting, threshing and further handling of seeds for grading and packaging.
(3.) Mutations: This is not a serious factor of varietal deterioration. In the majority of the cases it is difficult to identify or detect minor mutation.
(4) Natural crossing: In sexually propagated crops, natural crossing is another most important source of varietal deterioration due to introgression to genes from unrelated stocks which can only be solved by prevention.
Natural crossing occurs due to following reasons: Natural crossing with undesirable types, natural crossing with diseased plants and natural crossing with off- type plants.
Natural crossing occurs due to following factors: The breeding system of species, isolation systems,varietal mass pollinating agent, size of the pollen grains, and duration of pollen viability.
(5) Minor genetic variations: Minor genetic variations may exist even in the varieties appearing phenotypically uniform and homogeneous at the time of their release. During later production cycle some of this variation may be lost because of selective elimination by the environment.
(6) Selective influence of diseases: New crop varieties often become susceptible to new races of diseases often caused by obligate parasites and are out of seed programmes. Similarly, the vegetative propagated stocks deteriorate fast if infected by viral, fungal and bacterial diseases. During seed production it is, therefore, very important to produce disease free seeds/stocks.
(7) Techniques of plant breeders: In certain instances, serious instabilities may occur in varieties due to cytogenetical irregularities not properly assessed in the new varieties prior to their release. Other factors, such as break down in male sterility certain environmental conditions and other heritable variations may considerably reduce the genetic purity.

Maintenance of genetic purity during seed production: The various steps suggested by Hartmann and Kester (1968) for maintaining genetic purity. Minimum genetic purity standard is given in table-4.

a. Providing adequate isolation to prevent contamination by natural crossing or mechanical mixtures (Table-2).
b. Use of approved seed only in seed multiplication by adopting generation system (In India three generation system of seed production is followed i.e. starting from breeders seeds then foundation seed and then certified seed).
c. Rouging of seed fields, prior to the stage at which they could contaminate the seed crop.
d. Periodic field inspectionat critical stages for verification of genetic purity, detection of mixtures, weeds, and for freedom from noxious weeds and seed borne diseases etc. Avoiding genetic shift by growing crops in areas of their adaptation only (Table-2).
e. Certification of seed crops to maintain genetic purity & quality seed through seed certification agency.
f. Grow-out tests: This is mandatory for hybrids produced from hand emasculation and pollination method because there are chances of presence of female selfed seed.

(B) Agronomic principles-

1. Selection of agro-climatic region: Growth of the plant and production of good quality seeds are strongly influenced by both genetic and environmental factors. For good seed crop, a crop variety to be grown for seed production in an area where it must be adapted to the photoperiod and temperature conditions prevailing in that area.
2. Selection of seed plot: The plot selected for seed crop must be free from - volunteer plants weed plants, soil borne diseases & insects pests and have good soil texture and fertility.
3. Isolation of Seed crops: The seed crop must be isolated from- Other nearby fields of the same crop and the other contaminating crop as per requirement of the certification standards (Table-2).
4. Selection of variety: The variety of seed production must be adapted to the agro-climatic conditions of the region and it should possess some trait such asdisease resistance,earliness,grain quality and higher yield.
5. Seed treatment: Depending upon the requirement, seed should be treated by chemical (fungicide & insecticide), biocontrol agent (PGPRs) and by dormancy breaking chemicals.
6. Time of planting: The seed crops should invariably be sown at their normal planting time. Depending upon the incidence of diseases and pests, some adjustments, could be made, if necessary.
7. Seed Rate: Lower seed rates than usual for raising commercial crop are desirable because they facilitate rouging operations and inspection of seed crops.
8. Method of sowing: The most efficient and ideal method of sowing is by mechanical drilling.
9. Rouging: Adequate and timely rouging is extremely important in seed production. Rouging in most of the hybrid crops may be done at vegetative / pre-flowering stage, flowering stage and Maturity.
10. Supplementary pollination: Provision of honey bees in hives in close proximity to the seed fields of crops largely cross pollinated by the insects, ensure good seed set thereby greatly increase seed yields.
11. Weed control: Weeds may cause contamination of the seed crop, in addition to reduction in yield by enhancing competition.

12. Disease and insect control: Successful disease and insect control is another important biotic factor in raising healthy seed crops. Apart from reduction of yield, the quality of seeds from diseased and insect damaged plants is invariably poor.
13. Fertilizer application: In the nutrition of seed crops, nitrogen, phosphorus, potassium, and several other elements play an important role for proper development of plants and seed. It is, therefore, advisable to know and identify the nutritional requirements of seed crops and apply adequate organic fertilizers.
14. Irrigation: Irrigation can be important at planting for seed crops on dry soils to ensure good uniform germination and adequate crop stands. Excess moisture or prolonged drought adversely affects germination and frequently results in poor crop stands.
15. Harvesting of Seed crops: It is of great importance to harvest a seed crop at the time that will allow both the maximum yield and the best quality seed.
16. Storage of seeds: The hybrid seed of vegetables should be stored properly in air tight container after drying at optimum moisture content level. Optimum moisture content is given in table-3 as per IMSCS.

Table:2 Seed certification standards of different vegetable crops for hybrid seed production as per the IMSCS-2013. (FS-Foundation seed, CS-certified seed, FI field inspection)

Vegetable crop	**Isolation distance**		**No of FI**	**Stage of field inspection**
	FS	**CS**		
Brinjal	300	150	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Chilli	500	250	3	1st before flowering 2nd at flowering 3rd at mature fruit stage and prior to harvesting
Okra	500	250	3	1st before flowering 2nd at flowering 3rd at mature fruit stage and prior to harvesting
Tomato	200	100	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Bittergourd	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Bottlegourd	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Cucumber	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Muskmelon	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Watermelon	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting

Pumpkin	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Spounggourd	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Ridgegourd	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Summersquash	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Wintersquash	1500	1000	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Cauliflower, Broccoli, Knol-Khol	1600	1600	3	1st before flower stalk development 2nd during flowering 3rd at maturity and prior to harvesting
Onion	1200	600	3	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
True potato seeds	-	50		1st before flowering 2nd& 3rd at flowering 4th at harvesting
Carrot	1000	800	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting
Radish	1600	1600	4	1st before flowering 2nd& 3rd at flowering 4th at mature fruit stage and prior to harvesting

Table:3 Seed standards of different vegetables for hybrid seed production as per the IMSCS-2013. (GP%-germination percentage, PP-Physical purity, mc%- Moisture content, VPC-vapour proof container)

Vegetable crop	**GP%**	**PP%**	**mc%**	
			Max. mc%	**VPC%**
Brinjal	70	98	8	6
Chilli	60	98	8	6
Tomato	70	98	8	6
Okra	65	99	10	8
Bittergourd	60	98	6	7
Bottlegourd	60	98	6	7
Cucumber	60	98	6	7
Muskmelon	60	98	6	7
Watermelon	60	98	6	7
Pumpkin	60	98	6	7
Spounggourd	60	98	6	7

Ridgegourd	60	98	6	7
Summersquash	60	98	6	7
Wintersquash	60	98	6	7
Cauliflower, Broccoli, Knol-Khol	70	98	5	7
Onion	70	98	6	8
True potato seeds	80	98	6	8
Carrot	60	95	7	8
Radish	70	98	5	6

Hybrid seed production of vegetables

Seed production of vegetable can be categorized into self-pollinated, cross-pollinated open-pollinated, F_1 hybrid and clonally propagated cultivars. Among them the acceptance and demand of using F_1 hybrid in vegetable production is increasing rapidly due to their yield potential, resistance, quality attributes and storability. Hybrids varieties have been developed in those vegetable crops which manifest distinct hybrid vigour. Most of the seed of our main vegetables including tomato, chilli, brinjal, cucumber, squash, pumpkin, melon, watermelon, brassicas such as cabbage, cauliflower, broccoli, and radish, and onion are of F_1 hybrid cultivars. From the breeder point of view, it is a fast and convenient way to combine desirable characters of a vegetable together, for example fruit size and colour, plant type and disease resistance, and as a mean to control intellectual property rights through control and protection of the parental lines by the breeders. In F_1 hybrid vegetable seed production, vegetables can be divided into two groups: the hand-pollinated and the gene-control pollinated species. The genetic control system can be due to the self-incompatible system where pollen of the same plant or flower cannot pollinate itself or to the malesterile genetic system where a female plant has no male organ, deformed organ or no functional pollen to pollinate itself. When no such genetic control system is found or when it is not introduced into inbred parental lines, tedious hand-emasculation and pollination have to be used to produce F1 seed. In both the gene-control system and hand-pollinated species sufficient field or female flower isolation have to be maintained to obtain high seed genetic purity.

The success of hybrid vegetable technology primarily depends on genetic purity, timely availability and the affordability of hybrid seed costs to the farmers. The production of pure hybrid seed at affordable price in vegetables, is a highly skill oriented activity. A good hybrid may not reach a large number of farmers, unless it is feasible to commercially produce the seed on large scale economically. There are different methods for hybrid seed production in vegetables such as:

The gene-control pollination F_1 (hybrid) vegetable seed production system- There are two systems.

a. Self-incompatibility system based hybrids: These hybrids are developed for Crucifereae familyvegetables which includes *Brassica oleracea* (Brussel sprouts, cabbage, cauliflower, broccoli, kohlrabi and kale), *Brassica rapa* (Chinese cabbage, turnip and a range of Asian leafy brassicas) and *Raphanus sativus*. Maintenance of self-incompatible line is very difficult task. Different methods are available for temporary suppression of self-incompatibility *viz,*Bud pollination, CO_2 gas (CO_2enrichment) or sodium chloride, tissue culture using meristem, sodium chloride sprays, removal of stigmatic surface or whole stigma, high temperature treatment, and double pollination etc.

b. Male sterility based hybrids: Hybrid seed production of sweet corn, carrot and onion are based on male sterility gene system and the genetic control can be either just clear-cut male sterility genes or the interaction of a male sterility gene with a cytoplasmic factor. In this

system parents are maintained till foundation seed production by selfing or by crossing with maintenance line while for certified seed production male/restorer line and female parent are crossed to obtain hybrid seed.

The hand-pollinated F_1 (hybrid) vegetable seed production system

The method involves the manual emasculation of the pollen-producing organ (anthers) followed by hand pollination with pollen of the male parent and then preventing other pollen from contaminating the pollinated flowers. However, it is labour intensive and requires a team of skilful growers and many dedicate pollinators with good eye-sight, gentle hands, a lot of patience and commitment, and able to follow instructions accurately. To be cost-effective, this system only works in species where a single pollination of a female flower will produce many seeds e.g. solanaceous crops (tomato, brinjal) and cucurbits (bitter gourd, bottle gourd, summer squash, winter squash, pumpkin etc.).

Fig-1 Hand emasculation and pollination in tomato, Pollination in bitter gourd

Table-1 The most commonly utilized mechanisms/methods for developing commercial hybrids in vegetables(Kumar *et al.*, 2005)

Mechanism	Commercially exploited in:
Hand emasculation + HP	Tomato, eggplant, sweet pepper, okra, hot pepper
Pinching of staminate flowers + HP	Cucurbits (bitter gourd, bottle gourd etc.)
Male sterility + HP	Tomato, hot pepper, sweet pepper
Male sterility + NP	Onion, cabbage, cauliflower, carrot, radish, muskmelon, hot pepper
Self-incompatibility + NP	Most of the cole vegetables like broccoli, cabbage etc.
Gynoecism + NP	Cucumber, muskmelon
Pinching of staminate flowers* + NP	Cucurbits including bitter gourd, summer squash etc.
PGR and pinching of staminate flowers* + NP	Summer squash, winter squash etc.

HP = hand pollination; NP = natural pollination; PGR = plant growth regulator
* Genotypes with increased proportion of pistillate flowers are desirable for hybrid development.

Hybrid are advantageous over the varieties due to heterosis (high yield, earliness, quality) but hybrid seed production is laborious, time consuming and costly. It is not possible to develop hybrids in each crops. In legumes the small number of seeds per flower/pod and floral biology prevents hand-pollination to be efficient and thus no hybrid in pea and beans to date have been produced. In this case the use of gene-control pollination has to be exploited. Similarly, if a good gene-control pollination system is available in tomato and pepper their seed

production could be transformed into less intensive large field production system as in the brassicas and sweet corn.

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Seed Testing Methods in Vegetable Crops

Nakul Gupta, Shivam Kumar Rai, ChendraSekher and P.M Singh
ICAR-Indian Institute of Vegetable Research, Varanasi 221305, U.P.

Seed quality is a multiple trait which includes several components viz. purity (physical and genetic), germination, viability, vigour, freedom from other crop seeds, weed seeds and seed borne diseases, low seed moisture content and size. It is the degree of excellence in regard to the referred seed characteristics that determines the seed quality. Among all these parameters genetic purity and germination is most important as they determine the yield potential and field stand of any cultivar, respectively. Although the efforts are made to maintain the quality of seed during seed production, but still risk of contamination and deterioration of quality is there. Quality seeds make possible higher production and productivity of crop, avoid the spread of the disease form one place to another place, enhance the income of the farmer and stabilize any seed company in the market. Quality seed alone can lead to 15-20 % increase in productivity. Therefore, it is of paramount important to monitor and test the quality of the seed before its marketing and distribution to assure the quality of seed to the end users.

What is seed testing?

The seed testing is the science of evaluating the planting value of the seeds. Seed testing helps to assess the quality attributes of the seed lots which have to be offered for sale and minimizing the risk of planting low quality seeds.

Since seed is a global commodity, seed testing methods should be uniform in all over the world. International seed Testing Association (ISTA) 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. It also involves in ISTA Accreditation Programme, issuing Analysis Certificates exclusively by ISTA Accredited Laboratories (Orange International Seed Lot Certificate, Blue International Seed Sample Certificate), promotion of research, and training, publishing information in all areas of seed science and technology and cooperation with related organizations.

Objectives Seed Testing

- To determine the seed quality i.e., their sustainability for planting.
- To identify seed quality problems and their probable causes.
- To determine the labelling specifications.
- To establish quality and provide a basis for price and consumer discrimination among lots in the market.
- To determine the need for drying and processing.

Historical Events

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

1960	First Seed Testing Laboratory was established in India (CSTL at IARI, New Delhi)
1967	First seed testing manual was published for uniform testing.

Methods /procedures of seed testing

1. Seed sampling
2. Physical purity
3. Genetic Purity
4. Germination testing
5. Seed moisture testing
6. Seed health testing
7. Seed viability testing
8. Seed Vigour Testing

Seed sampling

Seed sampling is aimed at obtaining a sample of the required size and consisting of the same components as the whole lot of seeds. The quantity of seed tested in the laboratory is small, compared with the size of the seed lot, which it is intended to represent. No matter how accurately the laboratory work is done, the results can only show the quality of the sample submitted for analysis. Consequently, every effort must be made to ensure that the sample sent to the seed-testing laboratory accurately represents the seed lot in question.

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

Types of sample drawn at various stages

- *Primary sample:*It is the small portion taken from a lot at particular stage.
- *Composite Sample:* Formed by combining & proper mixing of all primary sample. Or pooled sample of all primary samples.
- *Submitted Sample:* sample which is submitted to seed testing laboratory and obtained from composite sample.
- *Working Sample:* Working Sample is a sub sample taken from Submitted Sample in the laboratory on which one of the quality tests is made.

There are three types of seed samples received by a seed-testing laboratory:

1) Service sample (2) Certification sample (3) Official samples

Service Samples means a sample submitted to the central seed testing laboratory or to a state laboratory for testing the results to be used as information for seeding, selling or labelling purposes. Any private individual i.e Farmer, seed dealer or any individual /agency can send sample to know the quality status of seed lot. The main purpose of analysing service sample is to provide results for sowing or labelling purpose.

Certification Sample: Certification sample means a sample of seed drawn by a certification agency or by a duly authorized representative of a certification agency established under section 8 or recognized under section 18 of the Seed Act 1966. The main purpose of such sample is to determine that sample in question confirms the prescribed quality standards for the purpose of seed certification or not.

Official sample means a sample of seed drawn by recognized official of seed law enforcement agency (Seed inspector) to ascertain that the seeds meet minimum limit of specified quality. All possible efforts are made to start testing a sample on the day of receipt. However, under unavoidable circumstances the sample shall be stored in dry cool, ventilated room so that changes in the quality of seed are minimized

The purpose of such sample is to know the quality status of a lot for the purpose of seed legislation.

(1) Physical purity testing

A purity test is conducted on approximately on 2,500 seeds which are broken down into four components: Pure seed, other crop seed, weed seed and Inert matter. The components are then weighed from which percentages are calculated. The percentage is based on the weight of each component, not the number of seeds.

Need for physical purity analysis

- Seed Certification or Seed Law Enforcement Agencies to judge that the seed lot conforms to the prescribed standards.
- Seed processing plants for using right kind of processing equipment.
- 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-

i) The percentage composition by weight of the sample being tested and by inference the composition of seed lot

ii) The identity of various species of seeds and inert particles constituting the sample.

Requirements for Purity analysis

- Purity table or working board,
- Seed divider,
- Forceps, Spatula,
- Brush Aluminum purity dish,
- Magnifier (5 to 7x),
- Analytical balance,
- Hand screen,
- Top loading balance,
- Stereoscopic binocular microscope,
- Seed blower,
- Table lamp,
- Watch glass

Components of Seed physical purity Analysis

- *Pure seed:* Seeds of kind/species stated by sander or found to predominant in the test.
- *Other crop seeds:* seeds of plants which are as crops, other than main crop.
- *Inert matter:* includes seed units and all other matter and structures not defined as pure seed, other crop seed or weed seed.
- *Weed seeds:* Seeds of a weed species which are recognized as weeds by law/general usage.

During purity analysis, each 'pure' seed fraction from the working sample is separated from the inert matter and other seeds. %age Weigh the 'pure' seed fraction should be express as the purity percentage.

Purity (%) = Weight of pure seeds (g) x 100 /Total weight of working sample (g)

Reporting of Results

- Weight by percentage Single decimal place
- All components should add to 100 %
- Less than 0.05% reported as 'trace'
- Any component is found to be nil- reported as '0.0'
- The components scientific names should be mentioned

(2) Genetic purity testing

Genetic purity or genuiness of the cultivar is tested by means of heritable characters (morphological, physiological or chemical) of seeds, seedlings or plants. The genetic purity can be

tested at seed level or field level. Following methods are used for testing the genetic purity of cultivar.

Genetic purity testing at field level- It includes morphological or conventional grow out test.
Genetic purity testing at seed level- It includes laboratory tests such as Chemical test, Biochemical markers-based test (proteins and isozymes) and molecular markers-based test. In this chapter chemical and biochemical tests are included.

- The test is based on appearance of seed as morphological character, seedlings or chemical test.
- The working simple consisted of 400 seed (4 x 100) taken randomly from sub-sample.
- The morphological characters are examined with the aid of a suitable magnification as length, breadth, thickness, size, shape, surface, volume, roundness, texture etc.
- The colour characteristics are examined under full daylight or ultraviolet light.
- Chemical characteristics are examined after the treating the seed with appropriate reagent and the reaction of each seed is noted.

A. Chemical tests for genetic purity testing:

The field testing has to wait thought out the growing season until all traits of the cultivar have been expressed. Hence, chemical tests are used as they are quick to establish the identity of a cultivar.
Some chemical test are as follows.

a. Phenol test: Varieties of cereals particularly wheat, rice and sorghum are verified based on activity of the enzyme tyrosinase using phenol as a substance (walls, 1965). It can be divided into a. Standard phenol test and b. Modified phenol test.

Procedure for standard phenol test

- Phenol solution (1.0%) is prepared by adding 5g phenol crystals to 500 ml distilled water. Store the phenol solution in an amber glass container in a cool place and do not use phenol solution which is more than three months old.
- Place two layers of filter paper in a Petri-dish and saturate with distilled water.
- Pour off excess water and place the seed on the filter paper (50 seed per Perti-dish of 9 cm diameter).
- Spread the seed uniformly on the surface of moistened filter paper.
- Cover the Peri-dish and incubate the seed overnight (18-24 hrs) at room temperature.
- Then add enough 1% phenol solution (about 3-5 ml per petri-dish) to moisten the filter paper.
- Close the petri-dish and after 4 hours record the colour reaction of seeds.
- The colour is observed as Nil or no reaction; Light brown; Brown; Dark brown; Black

b. Modified phenol test

The procedure is similar to standard phenol test but instead of distilled water the seeds are soaked in 0.4% of copper sulphate ($CuSO_4$) or 0.6% sodium carbonate (Na_2CO_3) solution and incubated overnight at room temperature. Then, add 1% phenol solution. After 4 hours the colour reaction is noted and observed. Based on the colour development the cultivars are categorized in different groups

c. Fluorescence test

Crops: Ryegrass, oat

Procedures:

- Place the seeds to be tested on a black background

- Evaluate the seeds for inflorescence under black light tubes in a room from which all other sources of light are excluded.
- Seeds are considered fluorescence of the lemma or pelea fluoresce or appear light in colour, partially fluorescent seeds should be considered fluorescent.
- Seed are considered non- fluorescent if the lemma and pelea do not fluoresce and appear dark in colour

d. Peroxidase test

Buttery and Buzzell (1968) separated soybean cultivar into two groups based on the presence of either low or high seed coat peroxidase activity.

Equipment and chemical: Guaiacols, hydrogen peroxide, test-tube or another suitable container

Procedure:

- Place seed coats removed from the soybean seeds into a test tube or suitable container
- Add 10 drops of 0-5% guaical solution to the test-tube
- After 10 minutes add a drop of 0.1% hydrogen peroxide solution
- One minute after adding the hydrogen peroxide, record the seed coat colour as peroxidase positive (high peroxidase activity) indicated by a reddish –brown solution or peroxidase negative (low or no peroxidase activity) indicated by colourless solution in the test tube.

e. Ferrous Sulphate ($FeSO_4$) test

Seeds are soaked in 1.5% solution of $FeSO_4$ for four hours in ambient conditions. Seeds and excess moisture is removed and evaluated for colour change as dark grey streaks, brown streaks, and brown spots on the seed coat. Since all the seeds are turned to grey colour, it was difficult to distinguish varieties. Hence, luminance meter is used to measure pixel luminance and plot a histogram based on the 8-bit gray scale. In a grey scale there are 255 different possible intensities and histogram displays 255 numbers showing the exact distribution of pixels among the grey colour samples. The gray scale measures pixel distribution from 0- 255, 0 is black, 128 is grey and 255 is white.

f. Sodium hydroxide test for wheat

A chemical test used to distinguish between red and white wheat. Particularly, when it is difficult to distinguish between the two types (i.e. when seeds are weather-damaged, treated, or are simply hard to distinguish by the naked eye).

Procedure-

- 100 seeds are soaked in three rep. in 5%NaOH solution for 1 hr at room temperature.
- Changes in the colour of seeds are observed after one hour.
- Based on the colour intensity of seed genotypes are classified into three groups which is dark brown, light brown and brown.

g. Seedling growth response to 2, 4-D

The reduction in coleoptile growth due to exogenous application of 2, 4-D is measured in this test. The seeds are surface sterilized by washing in distilled water. 100 seeds each in three replications are placed on two layers of blotter paper moistened with 10 ppm 2, 4-D solution and incubated at 25 ± 1 ℃ as per ISTA procedure. The water-soaked blotter papers were used as the control. On the 7th day, 25 seedlings are selected randomly, and growth response is measured regarding percent decrease in coleoptile length over that of control using the following formula.

Per cent decrease over Control = (Coleoptile length in control-Coleoptile length in 2, 4 D/Coleoptile length in control) x 100

h. KOH bleach test

This test is mainly performed for estimating genetic purity for sorghum crop. The presence or absence of a darkly pigmented testa can be used to differentiate sorghum cultivars. The dark pigment is tannic acid.

Procedure

- Prepare 1:5(w/v) solution of KOH and fresh bleach (5.25%NaOCl)
- Put seeds in glass container and completely cover with KOH bleach solution.
- Soak the seeds with brown seed coats for 10 min and seeds with white coat for 5 min.
- Gently rinse with tap water then air dried it.
- Record number of dark and light seeds and they are examined for presence of dark pigmented tannic acid.

i. Gossypol Test for cotton

Gossypol content of cotton is mainly dependent on genetic constituent of pigment glands. The glanded cotton normally contains gossypol in both seeds and plants.

Complex Reagent preparation:

2 ml of 3-amino propanol is added to 10ml of glacial acetic acid and this solution is then made up to 100 ml using N,N-dimethyl formamide. Solvent based method is the technique used for extraction of gossypol from cotton seed samples. Presence of gossypol in the solvent can be confirmed by yellow colour reaction.

Method of Gossypol analysis:

All the cotton seed samples are manually crushed followed by grinding samples into powder form. Extraction of total gossypol is carried out by N, N-di methyl formamide, during the process of reaction evaporation of N, N-di Methyl formamide is performed at high temperature till an oily material containing gossypol is obtained. Extraction of Free Gossypol is carried out by aqueous acetone.

Estimation of total gossypol:

For 1g of each sample, 25 ml of complex reagent is added and then placed in water bath at 100˚C for 2 hours. This process results in the formation of yellow colour indicating the presence of gossypol. After colour reaction is observed, samples are allowed to cool to room temperature upon which filtration was performed. Dilutions were performed by taking 1ml of filtrate to which 4 ml of N, N-di methyl formamide was added. 20µl of this sample is injected for HPLC analysis. As per amount of gossypol, we can determine the genetic purity of Cotton. Basically, this method is used to differentiate Bt- and Non-Bt cotton.

Advantages of chemical tests

- They are quick.
- They require virtually no technical expertise or training.
- Relatively inexpensive to conduct.
- No sophisticated equipment are required.
- The test permits detection of percentage admixture of other type.
- Its results are usually distinct and easily interpretable.

B. Biochemical tests for genetic purity testing:

Electrophoresis is a technique which separates mixture of protein into district bands in a gel that has been placed into an electric field. Each variety has a specific banding pattern on the basis of which admixture of other varieties, differing in banding pattern could be detected. Genetic purity test in many other crops has been investigated in several studies using isozyme polymorphism. Isozymes (proteins of similar molecular weight having different forms) are used for differentiation of parental lines and hybrids, e.g. APS, esterases, peroxidases, etc.

This procedure may be limited by environmental conditions and tissue type and may require selection of a suitable isozyme for purity testing. Isozymes were first described by R L Hunter and Clement Merkert (1957) who defined them as different variants of the same enzyme having identical functions and present in the same individual. Isozymes (also known as isoenzymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction.

An example of an isozyme is glucokinase, a variant of hexokinase which is not inhibited by glucose-6- phosphate. Its different regulatory features and lower affinity for glucose (compared to other hexokinases), allows it to serve different functions in cells of specific organs, such as control of insulin release by the beta cells of the pancreas, or initiation of glycogen synthesis by liver cells. Both of these processes must only occur when glucose is abundant, or problems occur.

Principle: the term of electrophoresis refers to the migration of a charge particle under the influence of an electric field. The movement of ions take place in a suitable medium such as polyacrylamide gel. Which is act as molecular sieve and cut down. This separation into distinct bands is due to difference in (a) size (molecule weight); small partials migrate faster than higher weight (b) and charge of the proteins involved.

There are various electrophoretic methods available:

- Acid-PAGE
- Alkaline- PAGE
- Sodium Dodecyl Sulphate Polyacryamide gel electrophoresis for denatured protein (SDS-PAGE)
- Polyacryamide gel electrophoresis (PAGE)

Steps

1. Sample preparation (Protein extraction)
2. Gel preparation
3. Electrophoresis
4. Staining and interpretation

Equipment: electrophoresis unit, electricity supply, micropipettes, Polyacryamide gel

C. DNA markers-based testing

DNA molecular technology, which are based on sequence variation of specific genomic regions, provide powerful tools for cultivar identification. Molecular marker is an identifier (sometimes called a "tag") of a particular aspect of phenotype and/or genotype; its inheritance can easily be followed from generation to generation. Molecular markers are classified in to four groups.

- Probe (Southern hybridization) based– Restriction fragment length polymorphism (RFLP),
- Amplification or PCR based- Random amplified polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR) and Sequence Characterized Amplified Region Markers (SCAR)
- Combination of probe based and PCR base markers- Amplification Fragment Length Polymorphism (AFLP)
- New generation markers – Single Nucleotide Polymorphism (SNP)

Advantages of molecular marker for genetic purity testing

- Accurate, rapid and reproducible cultivar identification
- A random scattering across the genome and established variation between the cultivar at the level of nucleotide sequences

- Great informative power
- Simple co-dominant inheritance
- Unaffected with respect to developmental stage, season, location and agronomic practices
- Potential for automation
- Less labour-consumption
- Conventional methods may fail to differentiate many vegetable cultivars because of their narrow genetic variation.

(3) Germination testing

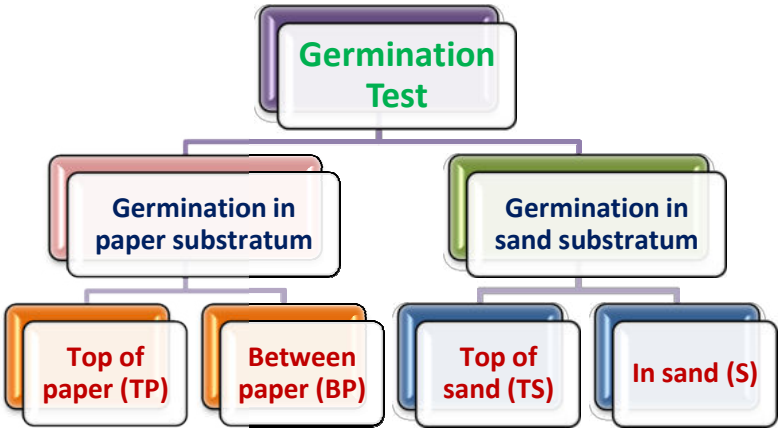
Seed germination is a physiological process, its consecutive numbers of step which causes quiescent seed with low moisture content to show a rise in its general metabolic activity and initiate formation of seedling from embryo.In other word "Seed germination is the resumption of active growth of the embryo that results in the rupture of the seed coat and the emergence of the young plant under favorable conditions."It is the emergence and development from the seed embryo of those essential structures which for the kind of seeds being tested, indicate the ability to develop into a normal plant under favorable conditions in the soil.

General Procedure for germination test:

- The working sample for germination test consists of 400 pure seeds randomly drawn either manually or with the help of counting device such as counting board or seed counter should be tested in replicates of 100 seeds.
- The seeds counted and evenly spaced on the germination substrate.
- Temperature must be maintained within 1°C of prescribed limit when using alternating temperature for germination e.g. in groundnut, the test is conducted at low temperature for 16 hrs and at high temperature for 8 hrs per day.

Methods for germination test:

There are mainly three methods for seed germination test based on substratum used.



Top of Paper (TP) method:

Procedure:

1. Cut the paper to the size and shape of the Petri dish or germination box or a tray fitted with lid.
2. Place a layer of paper in each Petri dish. If the paper is too thin use a double layer.
3. Label the top and bottom of each Petri dish with the accession number, number of the replicate and date of the test.

4. Moisten the papers with good quality water.
5. The thickness of paper used should be more than 2 mm when moist.
6. The water used must be reasonably free from acid, alkali, organic material or other impurities. It can be either tap, distilled or de-ionized water.
7. Seeds are placed directly on one or more layers of moist filter papers or blotters either in a petri dish, germination box or a tray fitted with lid to restrict moisture loss e.g. small seeded species.
8. Arrange the seeds in a regular equidistant pattern on the surface of the paper and placed inside the germination cabinet.
9. Add more water if required.

Advantage:
Seeds can be observed through the transparent lid and the germinating seeds are easy to count.

Disadvantage:
The petri dish soon dry out and need daily watering.

Between Paper (BP) method:
Procedure:

1. Cut the paper to a convenient size to hold one replicate of the seeds when spaced at regular intervals. If the paper is too thin, use a double layer.
2. Label each sheet on the outside of the paper at one end with the accession number, replicate of the test and the date of the test.
3. Moisten the paper with water.
4. Arrange the seeds at regular intervals on the paper, leaving at least two centimeters clear from the edges all rounds.
5. Leave enough space around the edges of the paper so that the paper can be folded back without damaging the seeds.
6. Cover the seeds with another sheet of paper and fold in the edges to prevent the seeds from falling out.
7. Normally 100 seeds are placed on two wet towels and one towel is placed over the seeds. Thus, the seeds are sandwiched between the paper towels.
8. Folding the edges is especially important for large spherical seeds which tend to fall out because of their weight and shape.
9. Roll the paper loosely towards the end with the label.
10. Do not roll the paper too tightly because the compression and lack of oxygen cause the seedlings to develop contorted roots and shoots.
11. The rolled paper towel is then kept in upright position in a germination chamber at a specified temperature for a specific period, which varies from species to species. If germination chamber is not available, then place the rolled papers inside ventilated plastic bags or boxes and put these in an upright position in wire baskets or plastic boxes at ambient conditions.
12. Oxygen is essential for respiration during germination. Therefore, any containers used should be adequately ventilated.
13. Keep the paper moist with water if necessary.

Advantage:

- The between paper method is cheap and easy to prepare the samples for germination test.
- The use of new paper at each test has the advantage that fungal contamination cannot be carried from one test to another test.

Disadvantage:
The seeds cannot be observed without unrolling the paper.

Germination in sand substratum:
Germination in sand is especially useful for large seeds which are too large to be germinated in petri dishes or too heavy for the between paper method. Sand should be washed, free from toxic materials and sterilized. The sand particle size should range between 0.05 to 0.8 mm in diameter. Generally, there are two methods using sand substratum:

- *Top of Sand (TS):* The seeds are pressed into the surface of the sand.
- *In sand (S):* The seeds are placed on a leveled layer of moist sand.

Procedure:

1. Pack clean sterile sand into post or trays with drainage holes at the bottom.
2. Water the sand until it is moist. Do not use excess water.
3. Fine sand should be used. Make sure that the sand is clean by sterilizing before use. Quarry or river sand is better than shore sand, which must be washed thoroughly to remove all the salts.
4. Make holes in a regular equidistant pattern at about the same depth to maintain uniformity of test for each replicate. Ideally, the distance between holes should be at least three to five times the seed diameter.
5. Prepare a label with the accession number, date of sowing and replicate of the test and place in each pot or tray.
6. Fill seeds from each replicate into the holes and cover with sand.
7. Water the sand again to cover the seeds, but do not make it too wet.
8. Sprinkle with water slowly, so that the seeds do not float out from the holes and become mixed. Bottom watering is better than top watering.

Seedling Evaluation:
The time of the first count is most appropriate but must be sufficient to permit the seedling to reach a stage of development so that accurate evaluation of seedling may be made. During first count, sufficiently well-developedseedlings are recommended to be counted in order to make evaluation easier and to prevent them from affecting the development of other seedlings. If the germination test is conducted in sand, the first count is omitted. If lower temperature is chosen, the first count should to be postponed. If the maximum germination of the sample is obtained before the end of prescribe test period, a test may be terminated.

On the request of the producer, the seed testing laboratory may release the result of seed germination test on the basis of first count if the sample meets the minimum prescribed germination standard for certification and labeling.Seedlings which have reached a stage when all essential structures isaccurately assessed shall be removed from the test at the first or any other intermediate counts. Badly decayed seedlings should be removed to avoid secondary infection to other seedlings but abnormal seedlings with other defects should be assessed only on the final count.

Types of seedling: The seedlings for germination test are classified into the following categories:

(1) Normal seedling:
Seedlings which show the potentiality for continued development into normal plants when grown in good quality soil and under favorable conditions of moisture, temperature and light are called normal seedlings. In addition, the normal seedlings must be conformed to one of the following categories.
Intact seedlings: Seedling with all the essential structures (like root system, shoot axis, elongated hypocotyls and epicotyls, etc) well developed and complete in all respect showing the proportionate growth and healthy are classified as intact seedling.
Seedlings with slight defects: Seedling which show certain slight defects in their essential structures (like primer root with limited damage or slight growth retardation, hypocotyl, epicotyl, mesocotyl, cotyledons, primary leaves/leaf, coleoptile with limited damage) provide they show an otherwise satisfactory and balanced development comparable to that of intact seedlings are classified as seedlings with slight defects.
Seedlings with secondary infection:
Seedlings which confirm either intact seedlings or seedlings with slight defects, but are affected by fungi or bacteria from sources other than the parent seeds are classified as seedlings with secondary infection.

(2) Abnormal seedling:
The seedlings which do not show the potentiality to develop into a normal plant, when grown in good quality soil and under favorable conditions of moisture, temperature and light are called abnormal seedlings because one or more of the essential structures (like primary roots, hypocotyls, epicotyl, mesocotyl, cotyledons, primary leaves/leaf, coleoptile, seedling as whole deformed, etc.) are defective. Abnormal seedling can be classified into the following types:

Damaged seedling: Seedling with any of the essential structures missing or so badly damaged that balanced development cannot be expected.
Deformed or unbalanced seedlings: Seedlings with weak or unbalanced development or physiological disturbances or in which essential structures are deformed or out proportion are called unbalanced seedlings. The damage to the embryo inside the seed is caused by external factors like mechanical handling, heat, drought or insect damaged which leads to such abnormalities.
Decayed seedling: Seedling with any of the essential structures causes diseased or decayed as a result of primary infection due to which normal development is prevented. These seedlings may result from external or internal seed borne diseases.
Ungerminated seed: Seeds which remain ungerminated by the end of germination test. This includes the following categories.
Hard seeds: Seeds which remain hard at the end of the prescribed test because they have not absorbed water due to an impermeable seed coat, e.g. Fabaceae, Malvaceae, Cucurbitaceae etc.
Fresh ungerminated seeds: Seeds other than hard seeds which absorbed water but not germinated and appeared firm or clean and remained apparently viable at the end of the test period.
Dead seeds: Seed which absorbed water but not germinated due to loss of viability. Such seeds are usually mouldy and soft in appearance.

Recording and reporting of germination test:

• The results of the germination test are recorded on the analysis cards of a suitable format. The seed analysts should interpret the result and decide whether the results to the four replications are within the permissible limit of tolerance before reporting the results.

• The result of germination test should be calculated on the basis of the percentage ofnormal seedling and should be reported in whole number. The percentage of hard seed should be reported separately. The percent of germination is calculated as under:

Germination % = Total number of seeds germinated/Total number of seed planted x 100

Determination of Seed Moisture Content

The amount of moisture in a seed has a direct impact on every physiological component of seed quality. It has been demonstrated that there are correlations between moisture content and seed maturity, ideal harvest time, storage longevity, cost-effectiveness of artificial drying, damage from heat, frost, fumigation, insects, and pathogens, mechanical damage, and seed weight. Seed moisture measuring techniques suitable for the purpose are required in both research and business because seed moisture and its management affect so many physiological quality variables crucial to quality control.

There is no one method of moisture determination that is suitable for all products or circumstances, as evidenced by the extensive literature on the determination of moisture content in a variety of items. The same is true for assessing the moisture content of grains and seeds. The most effective method for evaluating seed moisture depends on the chemical make-up and seed structure, moisture content level, level of accuracy and precision needed, and time, technical know-how, and cost restrictions.

Calculating the moisture content of seeds: The ideal device might be one that is applied to all seeds, measures moisture content from 0 to 100%, is repeatable, needs little training, and is inexpensive. One cannot blend all of these. However, techniques for determining the moisture content of seeds can be roughly divided into two categories:

1. Direct method 2. Indirect method

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

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

Indirect method

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

The Karl-Fisher method has been regarded as the most precise and fundamental reference method for establishing standards for other methods of determining seed moisture. The sole feasible technique recognized by the International Seed Testing Association (ISTA) and other

organizations for use in routine seed moisture analysis in a seed-testing laboratory is the constant temperature oven drying method.

Constant temperature oven drying method

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

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

Low constant temperature oven method:

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

High constant temperature oven method:

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

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

Apparatus: The following apparatus is required, depending on the method used: Grinding mill; Electrically heated oven; Containers; Desiccator;

The measurement of seed moisture should be done twice using two working samples that were drawn independently.

↓

With an accuracy of 1 mg or 0.1 mg, weigh each bottle.

↓

To start, weigh the container or bottle empty with its lid on.

↓

If necessary, ground the seed material.

↓

Small quantities (4 to 5 gm) of the seed samples from the sample should be evenly distributed on the bottom of the weighing bottles

↓

Remove the cap or lid from the weighing bottles or containers after you've finished weighing.

↓

Place the weighing bottles or containers in an oven that has been preheated to the desired temperature or is sustaining it for the specified time.

↓

Closing the lid or cover on the weighing bottles or containers after the seed drying period is complete.

↓

Transfer the weighing bottles and containers to desiccators with blue silica gel for cooling for 40 to 45 minutes.

↓

Reweigh the weighing bottles or containers after cooling.

↓

Determine the moisture content of the seed.

Procedure:

Calculation of results

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

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

Where,

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

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

M_3 is the weight of the weighing bottle or container after drying the seeds and adding the lid.

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

Seed Health

Seed health refers to the presence or absence of diseases causing organisms such as fungi, nematodes, bacteria, viruses and insects and to the status of seeds in a seed lot.

Seed status is also affected by the presence of non-disease-causing contaminants in the particular seed lot. These include contaminants like weed seeds that compete with the target seed for nutrients. Other seeds, plant parts other than the target seeds, soil particles and insect eggs that can overwinter can degrade the quality of the seed lot.

Seed health testing

Seed health testing is a procedure by which can be determined whether tile seed is healthy or diseased or it is a procedure by which the presence or absence of seed borne pathogen in a seed lot can be determined.

Objective of seed health testing

- Testing for Quarantine
- Testing for evaluation of planting value

- Testing for certification scheme

The measurement of seed moisture should be done twice using two working samples that were drawn independently.

↓

With an accuracy of 1 mg or 0.1 mg, weigh each bottle.

↓

To start, weigh the container or bottle empty with its lid on.

↓

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Small quantities (4 to 5 gm) of the seed samples from the sample should be evenly distributed on the bottom of the weighing bottles

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↓

Transfer the weighing bottles and containers to desiccators with blue silica gel for cooling for 40 to 45 minutes.

↓

Reweigh the weighing bottles or containers after cooling.

↓

Determine the moisture content of the seed.

- Testing for advisability of seed treatment
- Testing seeds for storage quality of for feeding
- Testing for resistance of cultivars

Location of Seed-borne Pathogen

⇒ Embryo
⇒ Endosperm
⇒ Seed coat
⇒ Surface borne

Methods of Seed health testing

1. Visual examination of dry seed
2. Seed washing test
3. Blotter method
4. Washing test
5. Agar plate method
6. Growing-on test

Dry Inspection method

It is a very simple and preliminary method for testing the seed health. The dry seed samples are examined for impurities such as Inert matter (plant debris, spotted, unfilled and Chaffey grains, galls, smut balls, insects etc).Seed samples are examined first by naked eye and the observed under a sterio binocular microscope for confirmation of the above impurities in proper way. The inert matters need to be incubated for the detection of pathogen associated with these.

Advantages

- It provides quick information.
- It does not require much equipment.
- It helps to take first hand measure.

Disadvantages

- Only those diseases giving external symptoms and sign can be detected by this method.
- Information pertaining to viability of seeds cannot be obtained.
- It is not more reliable because badly infected seeds may look healthy.

Seed washing test

Applicable solely for seed born fungal and bacterial pathogens. A known amount of seed in suspended in known amount of sterile saline (8.5% NaCl) overnight. Extract is plated on agar medium and incubated. Count number of colonies to determine CFU/seed for bacteria.

Blotter Method

The blotter test is a combination of in vitro and in vivo principals of investigation. In this method, the seed are sown in petridish or other suitable containers on moistened absorbent blotting paper, usually three layers to provide enough moisture for duration of the test.

Procedure:

- Required number of plastic petridishes was taken.
- The petridish were then rinsed with methylated spirit and were dried.
- The petridishes were left for a while to allow the spirit to be given off.
- Required number of blotting paper were soaked in sterilized water and three sterilized blotting papers were then placed on each petridish.
- Then 400 seeds were taken randomly from a working sample.

Surface sterilization of seedlot: Then the seedlot or working sample were sterilized with 0.001% $HgCl_2$ solution or 10% clorox solution for detection of internal seed borne pathogen.

Results:

After a week, incubated petridish containing seedlings and non-germinated seeds are carefully observed under steriobinocular microscope. The identification and the frequency of different category of fungi associated with the seeds were performed by observing the colour, growth habit and morphological features.

Advantages

- Pathogen can be detected quickly by observing their growth characters.
- It is economic.
- It can be applied for detecting wide range of fungal pathogens from all different kinds of seed.
- Results obtained by it, more reliable because it is the combination of vitro and in vivo.
- Blotter method is widely used while agar plate method is impracticable.

Disadvantages

- Examination may be hampered due to the first growth of certain fungi over the slow growing ones.
- Pathogenic bacteria cannot be detected.
- It is time consuming.
- Pathogenicity cannot be detected.
- Symptoms may not be detected.

Seed VigourTest

Seed vigour, a single concept reflecting severalcharacters determines the seed quality anduniform emergence potential of plants in fieldunder variable range of environments. It was in 1876, whenNobbe first used the term 'Seed Vigour',thereafter, seed scientists are constantly diggingin, to explore every possible scope this conceptcould provide. The mostacceptable definition evolved by the InternationalSeed Testing Association (ISTA) states that 'seedvigour is the sum total of all those properties thatdetermine the activity and performance of seedlots having acceptable germination in a widerange of environments. When the growingconditions are optimal, every viable seed couldgerminate except the hard or dormant one, but ifthe conditions are stressed and some seeds couldstill perform better than the rest, we need toaddress the concept of seed vigour.

Seed Vigour Evaluation

Seed vigour does not reflect a specific propertyof a seed or seed lot but it is still a concept.Several factors like genetic constitution, growthenvironment and nutrition of mother plant,maturity at the time of harvest, seed size andweight, mechanical stability, deterioration andageing and pathogens are responsible fordifference in seed vigour. There isno universal seed vigour testing method becauseseed vigour is influenced by multiple factors i.e.species, genotype and environmental conditions.It can be evaluated by various methods, such asvigour indices, stress tests like accelerated aging test, cold test, controlleddeterioration test, Brick gravel test etc. The mostwidely used methods are standard germinationtest and accelerated aging test.

A. Growth tests:

The basic principle behindthese tests is that seeds with high vigourgrow at a faster rate as compared to seedshaving poor vigour potential. This differencein growth can be easily observed even underfavorable conditions. Vigorous seedsmetabolize their food reserves rapidly,germinate, and establish in the field.Therefore, any method used to determine thequickness of growth of the seedling will givean indication of seed vigour level.

⇒ First count: The test is done along withthe regular standard germination test.Number of normal seedlings emerged onthe first count day, as specified for each species are counted. The number of normal seedlings gives an idea of the seed vigour potential in the seed lots. Higher the number of normal seedlings, greater is the seed vigour

⇒ Speed of germination: This test can be executed using either 'top of the paper or 'sand' method of the standard germination test. One hundred seeds each in four replications are planted for germination. The substratum is kept in a germinator maintained at recommended temperature for the crop. Number of seedlings emerging daily, are counted from day of planting till the completion of germination

⇒ Seedling length and dry weight: Theseedlings are grown either in laboratory,green house or field. In laboratory,between papers method should befollowed. Seeds are planted between twomoist towel papers in such a way thatthe micropyles are oriented towardsbottom to avoid root twisting. The rolledtowel papers are kept in the germinatormaintained at a

temperaturerecommended for crop in reference.After a specified period of time (according to reference crop), length ofemerged seedlings is measured, andmean seedling length is calculated. Seedlots producing the longer seedlings areconsidered more vigorous. For dryweight determination, the seedlings aretaken and dried in an air oven at 100°Ctemperature for 24 hours.

⇒ Seedling vigour indices: These indicesare given by Abdul-Baki and Andersonin 1973. These are derived from standardgermination and seedling growthparameters i.e. length and dry weight asper the following formulae:

i) *Vigour Index-I* = Germination (%) × Average Seedlinglength (cm)
ii) *Vigour Index-II* = Germination (%) × Average seedlingdry weight (mg)

B. Stress tests:

These tests are based on theassessment of seed performance understressed conditions. Seeds with higher vigourpotential perform better than low vigourseeds when tested under unfavorableconditions i.e. high or very low temperature,high humidity, high moisture content, somephysical barrier etc.

a) Hiltner Test (Brick gravel test): This test was developed by Hiltner in Germany in1917. He observed that *Fusarium* affected seeds of cereal crops were able to germinate in regular test but were not able to emerge from brick gravels of 2-3mm size. On the contrary, healthy seeds were able to emerge from the brick gravel. The underlying principle is that the weak seedlings are not able to generate enough force to overcome the pressure of brick gravels, so this method can be used to differentiate vigour levels in cereal seeds. The procedure involves the following steps:

The sand is sieved, moistened and filled in the germination box leaving about 3 cm empty at the top.One hundred seeds are placed in eachbox in the impressions made by a sandmarker. After this 2-2.5 cm of porousbrick gravel is spread over the seeds.The box is kept in the germinator atappropriate temperature. After theperiod required for germination, theseedlings which have emerged throughthe brick gravel layer are counted andpercentage of emerged seedlings areused to compare seed vigour of differentlots.

b) Paper Piercing test: The principle ofpaper piercing test is similar to that ofbrick gravel test. High vigour seed lotsare expected to produce strong seedlingswhich can pierce a particular type ofpaper while seedlings of poor vigour lotsmay not be able to pierce the paper.

c) Cold Test: Cold test was developed inUSA to evaluate the seed vigour ofmaize. In USA, when the corn is plantedin late spring, the soil is humid and cold.The weak seeds do not germinate andestablish. Therefore, to simulate theactual field conditions cold test has beendeveloped. This test aims to differentiatebetween weak and vigorous seed lots bysubjecting them to low temperature priorto germination at optimum temperature.The procedure involves following steps:

After grinding and properly sieving thesoil is filled in tray upto 2 cm depth. Fiftyseeds are placed over the sand andcovered with another 2 cm thick layer ofsoil. The soil is compacted, and enoughwater is added to make the soil saturateits water holding capacity. Afterwatering, the trays are covered withpolythene bags and placed in therefrigerator maintained at 10°Ctemperature for one week. After oneweek, the trays are placed in thegerminator at 25°C temperature. Theseedlings emerged after 4 days arecounted and germination percentage iscalculated by counting the number ofnormal seedlings. Higher thegermination percentage greater is thevigour.

d) Accelerated Ageing (AA) test: Theaccelerated ageing test was developed atthe Seed Technology Laboratory,Mississippi State University, USA fordetermining the storage potential of seedlots. The ageing process is acceleratedby subjecting the seeds to hightemperature (40-50°C) and humidity(100%) in a chamber before standardgermination. The seed lots that showhigh germination are more vigorous andexpected to maintain high viabilityduring storage.

e) Controlled Deterioration (CD) test: Asthe name suggests, the test involves thedeterioration of samples of seeds fromseed lots in a precise and controlledmanner at an elevated moisture content(dependent on the species, often 20%)and temperature (45°C) for a definedduration.

C. Biochemical tests:

a) Tetrazolium (Tz) test: Tetrazolium is arapid test to estimate seed viability andvigour based on color alterations ofseed's living tissues in contact with asolution of 2,3,5 triphenyl tetrazoliumchloride, thus, reflecting the degree ofactivity of the dehydrogenase enzymesystem closely related to seed respirationand viability. Dehydrogenase isrespiratory enzyme whose activityproves that cell is alive. The reduction ofTTC (colorless) to Formazan (red) isthe responsible chemical reaction.Usually, this test is considered as aviability test but its results can also beinterpreted to estimate the vigour of theseed. Vigorous seeds show dark stains ascompared to seeds having poor vigour.

b) Electrical Conductivity (EC) test: Weakening of cell membrane in lowvigour seeds causes the leakage of water-soluble solutes like sugars, amino acids,electrolytes etc. when immersed indistilled water. EC is negativelycorrelated with the quality of seeds. Theconductance of sample is measured aftermeasuring the conductance of distilledwater. The reading of distilled water issubtracted from the sample reading toget the EC of leachates. The value is thencorrected for the temperature andmultiplied by the cell constant factor.The reading is expressed as desi-Siemens or micro Siemens/cm/g of seed.Lower the value of EC, greater is theseed vigour.

General Principles of Vegetable Seed Production

P.M. Singh
ICAR-Indian Institute of Vegetable Research, Varanasi

In India, about 11.3 million ha of land is under vegetables producing nearly 204 million tons. Due to fast changing dietary habits and awareness about nutritional security, the demand and in turn the area under vegetables, is expected to increase. There would be a tremendous emphasis on the availability of high quality seed of vegetables. 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. Although India ranks second in vegetable production, the quality vegetable seed production in the country has so far been insufficient. 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.

The growth of plant and the quality of seed production are strongly influenced not only by 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, rouging, harvesting and post harvest 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.

Isolation Distance

Maintaining proper isolation distance is a must for obtaining pure seeds of any particular variety. The isolation distance is decided based on the nature of pollination in a particular crop and the class of seed being raised. The recommended isolation distances for different vegetables are provided in the following table :

Table 1 : Recommended isolation distance for different vegetables

	Vegetable Crops	Minimum isolation distance (metres)	
		Foundation seed	Certified seed
1.	Cowpea	10	5
2.	Garden pea	10	5
3.	Chilli & Capsicum	400	200
4.	Cauliflower, Cabbage & Knol khol	1600	1000
5.	Lettuce	50	25
6.	Carrot	1000	800
7.	Onion	1000	500
8.	Radish and Turnip	1600	1000
9.	Okra	400	200
10.	Tomato	50	25
11.	Brinjal	200	100
12.	Cucurbits	1000	500
13.	Spinach beet (Palak)	1600	1000

Cultivation Practices

Solanaceous Crops

The method of cultivation for seed production is nearly the same as that of cultivation for vegetable production. However, the care must be taken to maintain the specified isolation distance. The details about sowing and transplanting time are given in Table-2 and seed rate, planting distance & fertilizers in Table-4. Roguing of seed crop throughout the crop period is must to maintain true to the type plants. The most important rouging stages have been given in Table-5.

Cole Crops

Individual plants with good curd (cauliflower), head (cabbage) or knob (knol-khol) are marked and allowed for bolting and seed production. Rouging of seed crop throughout the crop period is essential to maintain the true to the type plants (Table-5).

In case of cauliflower, the seeds of early and mid varieties are produced in plains while seed of late variety (snow ball types) are produced in mid hills due to low temperature requirement (below 10°C for 6–8 weeks after full vegetative growth, but before curd formation). The seeds of cabbage and knol khol are produced in high hills as they require chilling temperature of 4–7 °C for 6-8 weeks after head and knob formation to produce flower stalk.

The methods of seed production in cole crops are as follows:

I. Seed to seed method - In this method, the plants with good curd (cauliflower) head (cabbage) and knob (knol-khol) are left in the field where they flower and produce seeds. But this method occupies lot of area because selected plants for seed production are left scattered in the field. However, in early cauliflower this method yields relatively better quantity and quality of seeds. The seeds ripen from March to May.

II. Head to seed method - In this method, the selected plants with head of cabbage/curd of cauliflower/ knob of knol khol are uprooted carefully and replanted in a compact block for seed production.

Table-2 : Time of nursery sowing and transplanting in transplanted seed crops

S. No.	Name of the vegetable	Time of nursery sowing	Time of Transplanting
1.	**Tomato**	September	October
2.	Brinjal	July	**August**
3.	Chilli	July	August
4.	Cauliflower		
	Early	June –July	July-August
	Mid	August-September	September-October
5.	Onion		
	Kharif	June	August (Bulbs are harvested and planted in November)
	Rabi	November	January (Bulbs are harvested in April-May, stored for about 5-6 months and planted in November)

Onion

Onion seeds are largely produced in Maharashtra, Gujarat, Madhya Pradesh and Rajasthan. In Northern India, generally in U.P., Haryana, Punjab, Bihar it is suffering with Purple Blotch thus the seed yield is very low and uneconomic. It is largely a cross-pollinated crop with upto 93% natural cross pollination (NCP), but some self pollination does occur. It is chiefly pollinated by honeybees. For seed production, an isolation distance of 1000m for foundation and 500m for certified seeds must be maintained. The details about sowing and transplanting time are given in Table-2 and seed rate, planting distance & fertilizers in Table-4. It is a biennial crop and takes two full seasons to produce seeds. In the first year bulbs are produced and in second year seed stalks are produced. It requires cool condition during early development of the bulb crop and again prior to and during early growth of seed stalk. Varieties bolt readily between 10-15^{0}C.

Mostly bulb to seed method is used for seed production. Seed to seed method is used in case the bulbs have lower keeping quality or in unavoidable circumstances. Well matured bulbs should he harvested and topped leaving an 1/2 inch mark. Before storage a thorough selection and curing of bulbs should be done. The length of time required for curing depends on weather conditions and may take three to four weeks. The mature bulbs should be stored in well ventilated, cool stores (0-4.5^{0}C) until three to four weeks prior to planting. The roguing is started in the field when bulbs are not harvested. After harvesting the true to type bulbs are selected.

Peas and Beans

The agronomic practices for the cultivation of seed crop are more or less the same as for green pods except that the sowing is adjusted such so that the seed maturity coincides with the drier part of the season. The other details are provided in Table-3 & Table-4. Although a self pollinated crop, pea is well known for producing off type plants. Hence, rigorous roguing must be undertaken at flowering and fruiting stage as mentioned in Table-5.

Table-3 : Time of sowing in direct sown seed crops

Sl. No.	Name of the vegetable	Time of sowing
1.	Okra	a) Feb-March for summer b) June-July for kharif
2.	Peas	1st fortnight of November
3.	French bean	October last week and November 1st week
4.	Cowpea	July-August
5.	Radish	October
6.	Carrot	October
7.	Spinach beet	October- November
8.	Methi	October-November
9.	Cucurbits	a) Feb-March for summer b) June-July for kharif

Okra

The agronomic practices are almost same as to be followed for the crop raised for vegetable production. However, the attention must be paid to maintain the specified isolation distance. YVMV is a very common problem in okra. The affected plants should be rogued out in addition to roguing off type plants, as mentioned in Table-5. In the recent past okra leaf curl, another viral disease, is affecting okra in several parts of the country particularly under north Indian conditions. The care must be taken to rogue out the affected plants as soon as they are spotted.

Cucurbits

The method of cultivation for seed crop is same like growing for fresh fruits. The details are provided in Table-3 & Table-4. Roguing of seed crop (Table-5) throughout the crop period is a must to maintain the true to the type plants. It is better to train the plants on a bower for increased yield.

Table-4 : Seed Rate, Planting Distance and fertilizer application in different vegetable crops

Crop	Seed rate/ha	Sowing/ Transplanting Distance (cm)	Fertilizers ($N:P_2O_5:K_2O$) kg/ha	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	60x30	120:80:80	20kg/ha borax & 20kg/ha calcium carbonate
Peas	100-160kg	23x5-7	60:60:60	
French Bean	50kg (Bush type) 40kg (Pole type)	45x15 (Bush type) 60x30 (Pole type)	120:60:60	
Cowpea	20kg (Bush type) 15kg (Pole type)	45x15 (Bush type) 60x30 (Pole type)	40:60:60	
Okra	18kg (Summer) 10kg (Rainy)	60x15 60x30	120:60:60	
Cauliflower	600g (Early) 400g (Late)	40x30cm (Early) 60x45cm (Late)	120:60:60	25kg/ha borax & 1.5kg/ha ammonium molybdate
Cabbage	600g (Early) 400g (Late)	40x30cm (Early) 60x45cm (Late)	150:60:60	
Radish	8-10kg	Sowing 45x8 cm Stecklings 75x60 cm	100:50:50 100:80:60	10kg boron/ha
Carrot	8-10kg	Sowing 50x10 cm Stecklings 75x60 cm	80:60:80 60:60:80	
Onion	10kg	45x30 cm (Bulb spacing)	100:60:60	
Bottle gourd	4 kg	5x1m	80:60:60	
Bitter gourd	5 kg	2x0.5m	80:60:60	
Sponge gourd	4 kg	5x1m	80:60:60	
Cucumber	3 kg	2x0.5m	80:60:60	
Muskmelon	3.5 kg	2x0.3m	80:80:60	
Watermelon	4 kg	2x0.5m	80:80:60	

Root Crops

The seeds of Asiatic varieties of root crops are produced in the plains while the seeds of temperate varieties are produced in hills. The method of crop raising for seed is nearly the same as for fresh roots. However, the seed can be produced by seed to seed method or transplanted root to seed method. Transplanted root to seed method is better since it gives an opportunity to rogue out off type roots at the time of transplanting thus maintaining only true to the type plants for seed production. In root to seed method fully developed marketable size roots are uprooted, true to the types are selected from them and the stecklings are prepared from selected roots by cutting them in such a way that the plants with only 2-3 inch size above and lower

ground parts remain. These stecklings are then transplanted in a compact block as per the details given in table-4.

Harvesting, Seed Extraction and Seed Yield

Solanaceous Crops
Individual plants with good fruiting should be marked and ripe fruits be collected for seed purpose.

In tomato, the extraction of seed from ripe fruit is done by fermenting the crushed fruits for 1-2 days and then washing with water so that the seeds settle down and pulp and skin float which are easily separated. Seed separation can also be done using commercial grade HCl @ 100 ml per 10 kg of tomato pulp. It takes only about half an hour's time after which the seeds are cleaned up and dried to specified moisture content (Table-6). The quantity of fruit required to produce 1 kg of tomato seed varies from 160-210 kg depending on the variety. On an average 100-150 kg/ha tomato seed can be obtained.

In brinjal, the ripe fruits turned yellow are cut into pieces, water is added and allowed for fermentation. Seeds thereafter are washed, sieved and dried. About 200-300 kg seeds can be obtained from a hectare of brinjal crop. Acid method can also be used for seed extraction in brinjal.

In chilli and capsicum, the ripe fruits are harvested and dried. Drying can be done by spreading the fruits under the sun which may take 10-15 days time depending upon the light intensity or the fruits can be dried in hot air oven at about 54.4°C in 2-3 days. The seeds are extracted by breaking open the dried fruits by hand. An axial flow vegetable seed extracting machine can alternatively be used for seed extraction from tomato, brinjal and chilli fruits. The machine can extract tomato seeds @1.25 kg per man hour, brinjal @ 1.8 kg per man hour and chillies @ about 3.0 kg per man hour. On an average about 200-300 kg/ha of seeds in chilli and about 100-150 kg/ha of seeds in capsicum can be obtained.

Peas and Beans
When almost 90% pods on the plants mature and turn dry, the whole plants are uprooted and collected on the threshing floor. After about a week the seeds are separated out from the pods by threshing and winnowing. The ripe and dry pods can also be picked up by hand and threshed on small scale. Usually the moisture content of seeds at this time is higher therefore the drying must be resorted to maintain the specified moisture content of these crops (Table-6). On an average, from a hectare of seed crop about 1500 kg pea, 1000 kg French bean, 1000 kg cowpea, 700 kg Dolichos bean and 700 kg cluster bean seeds can be obtained.

Okra
The okra fruits are harvested when they become dry on the plant. Care should be taken to harvest the fruits before splitting starts at the ridges on the pods. If splitting takes place the seeds become exposed which affects their quality. The seeds are taken out from the pods by opening the dried pods. The seeds are dried to specified moisture level, cleaned, treated and stored. For one year storage 21°C temperature and 12% humidity should be maintained. An hectare of okra crop gives about 1000-1200 kg seeds/ha.

Cole Crops
The seed crop should be harvested when most of the pods turn yellow. After that it is dried and seeds are threshed out from the pods. On an average, seed yield of 300-400 kg/ha from cauliflower, 400-500 kg/ha from cabbage and about 400 kg/ha of knol khol can be obtained.

Root Crops

The seed crop of radish should be harvested when most of the pods turn yellow. After that it is dried and seeds are threshed out from the pods. A hectare of radish seed crop yields 600-1000 kg seed.

In carrot the seeds are formed in umbels. The first and largest umbel is formed on main flowering stalk and known as primary or king umbel. Secondary umbels are formed at the terminus of branches from the main flowering stem and flower in a sequence from the top to the bottom of the inflorescence. Tertiary umbels originate on secondary umbel stem. The seeds from primary umbels are heavier, more mature and of high quality. In carrot, all the umbels do not mature together therefore harvesting is done in two three times. However, the seed crop can be harvested when all the secondary umbels mature and tertiary umbels turn yellow. A hectare of carrot seed crop yields 450 to 500 kg seeds.

Cucurbits

The harvesting is done when fruits are fully mature or become dry. Generally, the seeds are extracted by cutting open the fruits longitudinally. In watermelon, pumpkin etc. seeds are embedded in the pulp and for these different methods are applied for seed extraction :

a) Mechanical methods - In this method machines like Axial Flow Vegetable Seed Extractor are used to separate out the pulp from seeds.

b) Chemical methods - In this method commercial HCl is used to separate the pulp from seeds within 15-20 minutes. Thereafter the seeds are washed in water and dried to prescribed moisture levels.

From a hectare of seed crop, 300-500 kg bottle gourd seeds, 100-300 kg bitter gourd seeds, 300-400 kg Luffa, pumpkin, cucumber & round melon seeds, 200-300 kg muskmelon seeds and 400-500 kg watermelon seeds can be obtained.

Onion

Seed is ready for harvest when first formed seed in the heads get blackened. During harvesting and curing of the seed fairly high temperatures and low humidity is desirable. Two to three pickings may be necessary to harvest the heads. Seed heads are cut, snapped off, keeping a small portion of the stalk attached. Seed heads after harvest should be thoroughly dried. Seeds from heads are removed by flailing. Seeds are then cleaned by putting them in water and then dried under sun or by drier and stored (The moisture content should not be more than 6-8 %). Seed yield is 850 - 1000 kg/ha.

Table-5 : Important rouging stages & permitted off types for production of differnt vegetable seeds.

Vegetable Crops	Minimum number of field inspections and roguing stages	Off type (%) Maximum permitted	
		F	C
1. Cowpea	2: From flowering to fruiting	0.10*	0.20
2. Garden pea	3: First before flowering, second at flowering and third at edible pod stage	0.10*	0.50*
3. Chilli & Capsicum	3: First before flowering, second at flowering and third at the mature fruit stage	0.10*	0.20*
4. Cauliflower, Cabbage & Knol khol	4: First before the marketable stage, second start of curd head formation, third when most plants have formed curd and fourth at flowering stage	0.10*	0.20**

5.	Lettuce	3: First before full grown stages in non-heading types, second full grown stage in non-heading type and third at flowering	0.10*	0.20*
6.	Carrot	4: First 20-30 days after sowing, second at lifting and replanting, third at flowering and fourth at maturity to verify the true nature of umbels	0.10*	0.20**
7.	Onion	4: (When seed crop is raised by the transplanting method) First early (20-30 days after sowing), second when bulbs are lifted, third when bulbs are replanted and fourth at flowering	0.10**	0.20**
		3: (When seed crop is raised by seed to seed method) First 20-30 days after sowing, second when bulbs are formed and third at flowering		
8.	Radish and Turnip	3: First 20-30 days after sowing, second when lifted and replanted and third at flowering	0.10**	0.20**
9.	Okra	3: First before flowering, second at full flowering and fruiting and third at mature fruit stage.	0.10**	0.20**
10	Tomato	3: First before flowering, second during flowering and the immature fruit stage and third at mature fruit stage	0.10*	0.20*
11	Brinjal	3: First before flowering, second during flowering and the immature fruit stage and third at mature fruit stage	0.10*	0.20*
12	Cucurbits	3: First before flowering, second during flowering and the immature fruit stage and third during mature fruit stage	0.10**	0.20**
13	Spinach beet (Palak)	3: First at cotyledon leaf stage, second during vegetative growth, third at bolting and flowering	0.10**	0.20**

* Maximum permitted at the final inspection; ** Maximum permitted at and after flowering; F= Foundation seed; C = Certified seed.

Table-6 : Seed Standards for Vegetables

Crop	Class of seed	Germination (%) (Min.)	Pure seed (%) (Min.)	Inert matter (%) (Max.)	Other crop seed Max. (no/kg)	Weed seed Max. (no/kg)	Moisture (%)	
							Ordinary pack	Vapour proof pack
Tomato	FS	70	98	2	5	None	8	6
	CS	70	98	2	10	None	8	6
Brinjal	FS	70	98	2	None	None	8	6
	CS	70	98	2	None	None	8	6
Chillis Capsicum	FS	60	98	2	5	5	8	6
	CS	60	98	2	10	10	8	6
Okra	FS	65	99	1	None	None	10	8
	CS	65	99	1	5	None	10	8
Cabbage	FS	70	98	2	5	5	7	5
	CS	70	98	2	10	10	7	5
Cauliflower Knol Kkol	FS	65	98	2	5	5	7	5
	CS	65	98	2	10	10	7	5
Radish	FS	70	98	2	5	10	6	5
	CS	70	98	2	10	20	6	5
Carrot	FS	60	95	5	5	5	8	7
	CS	60	95	5	10	10	8	7
Beet	FS	60	96	4	5	5	9	8
	CS	60	96	4	10	10	9	8
Spinach	FS	60	96	4	5	5	9	8
	CS	60	96	4	10	10	9	8
Amaranthus	FS	70	95	5	5	10	8	6
	CS	70	95	5	10	20	8	6
Lettuce	FS	70	98	2	None	5	8	7
	CS	70	98	2	None	10	8	7
Onion	FS	70	98	2	5	5	8	6
	CS	70	98	2	10	10	8	6
Garden pea	FS	75	98	2	None	None	9	8
	CS	75	98	2	5	None	9	8
Cowpea	FS	75	98	2	5	5	9	8
	CS	75	98	2	10	10	9	8
French bean	FS	75	98	2	None	None	9	7
	CS	75	98	2	None	10	9	7
Cucurbits	FS	60	98	2	-	-	7	6
	CS	60	98	2	-	-	7	6

SEED ENHANCEMENT TECHNIQUES IN VEGETABLE CROPS

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

Seed enhancement is a range of treatments of seeds that improves their performance after harvesting and conditioning, but before they are sown. They include pelleting, encrusting, film-coating, priming, hardening, pre germination, tagging and others, but excludes treatments for control of seed borne pathogens (Black & Halmer, 2006).

Objectives

- To improve germination/seedling growth through manipulation of seed germination / vigour (priming, hardening, pre germination, antioxidants)
- To facilitate seed planting (pelleting, coating)
- To remove weak or dead seeds using 'upgrading' techniques (density, color, sorting, x-ray).
- To deliver materials (other than pesticides) needed at sowing (e.g. nutrients, inoculants)
- Tagging of seeds with visible pigments or other materials / markers for traceability and identity

Priming

Seed priming is the treatment of seeds in which they are hydrated sufficiently to allow the preparative events for germination to take place but insufficiently hydrated to allow the radicles to emerge followed by drying before actual sowing. The priming of seeds advances and synchronizes germination, resulting in earlier and uniform seedling growth. The optimal priming effect is often obtained at the least negative water potential [ψ] that prevents radicle emergence. The degree of enhancement from priming depends upon following factors:

- Initial quality of the seed.
- Species being treated.
- Treatment conditions such as temperature, water potential (ψ), duration and other conditions specific to the priming medium.

Methods of Priming

1. **Uncontrolled Water Uptake**
   When water is used, the duration of priming and not the water potential of the solution prevents germination during treatment.

2. **Controlled Water Uptake**
   - **Osmotic Priming**
     Seeds are hydrated in liquid solution. A number of osmotica including PEG, Glycerol, Mannitol, KNO_3, KH_2PO_4 and other salt solutions have been successfully used.

- **Solid Matrix Priming**
  Seeds, a solid carrier material, and water are incubated together in a sealed container for a prescribed period of time. The mixture is dried and seeds are separated from the media and cleaned. Materials such as vermiculite, synthetic calcium silicate, calcited clay, sphagnum moss, shale and bituminous charcoal have been widely used for SMP.

- **Drum Priming**
  Seeds are hydrated to a pre determined water potential over a 24h period by placing them inside a horizontal rotating drum into which water vapour is released. The drum is mounted on an electronic balance connected to a computer to continuously monitor the level of hydration. After treatment the seeds are redried to their original moisture content.

Seed priming virtually arrests the seed germination after phase-II of the triphasic pattern of water uptake. Major metabolic events occur at this time to prepare the seed for radicle emergence. Bringing about all the seeds to a uniform physiological and metabolic state during the phase II enable maximum uniformity among the seedlings. Osmotic potential and duration of the priming contribute to the improved germination performance. Manipulations in metabolic changes during priming play a beneficial role in radicle emergence upon rehydration. The consequences of successful priming treatments show a rapid resumption of growth processes and improved germination.

Drew *et al*. (1997) reported that slow germinating seed lots of carrot, leek and onion primed in PEG, germinated immediately, dried back and stored for 6-12 months, get benefited more due to priming in terms of mean germination rate and time to 50% germination than faster ones. However the storage potential of primed seed is severely limited. Osmo priming with 2% KNO_3 at 20^{O}C for 6 days and hydro-priming (30^{O}C, 18hr) increases germination as well as improved radicle emergence and hypocotyl growth under temperature and osmotic stress in watermelon (Demir *et al*., 1999).

Germination performance is a result of alterations in energy metabolism and various biochemical and molecular processes during priming of seeds. Kang *et al*. (1997) reported that pepper seeds primed with K_3P0_4 showed improved germination rate and reduced days to 50% germination associated with the increased activities of aldolase, isocitrate lyase, isocitrate dehydrogenase and malate dehydrogenase.

Effects of Priming

- Increased rate of germination.
- Greater germination uniformity.
- Increased germination percentage.
- Wider temperature range for germination.
- Advancement of maturity.
- Weakening of barriers to embryo growth.
- Increased protein synthesis.

How Priming affects Seeds

- Developmental Advancement.
- Effects on Germination Temperature.
- Metabolic Repair Processes.
- Changes in seed morphology.
- Protein and DNA Synthesis.
- Changes in Seed Water Relations.

Pelleting and Coating

Pelleting is typically used to round out small or irregular shaped seed, or to make small seeds larger for improving singulation and speed of sowing. Coating (encrusting) applies less material, so the original seed shape (more or less) is still visible. Seed pelleting/coating aims to influence the external physical properties of the seed, affecting the sowing characteristics only. The stress of the coating process should not affect seed quality. By itself an ideal coating would be neutral in its influence on the speed, uniformity and percentage of germination when compared to the original raw seed lot. It would perform in the same manner as the raw seed under a wide range of environmental conditions: light, moisture, temperature, pH, soil type etc.

Method of Coating

Seed coating relies on technology developed by the pharmaceutical industry to make medicinal pills.

- Seeds in a rotating drum are misted, and blends of powdered materials (e.g. chalk, clays, perlite, lime, peat, talc) are progressively added, along with more water, until desired pellet wt. or size increase is achieved.
- Each misted seed becomes the center of an agglomeration of powder that gradually increases in size. The pills are rounded and smoothed by the tumbling action in the pan.
- Binders are often incorporated near the end of the coating process to harden the outer layer of the pill. Binders can also reduce the amount of dust produced by the finished product in handling, shipping and sowing.
- Tumbling action distributes blending material to give good size distribution and prevents formation of empty (seedless) pellets or seeds sticking together.
- Pelleting increases seed wt by ~2 to 50 times (or more in tobacco seed); compared to an increase of only 0.1 to 2 times for coatings/encrusting, and less than 0.1 times increase for film coating.

Pelleting process can be broadly classified into three parts:

(1) stamping
(2) coating
(3) rolling

Seeds are first mixed with adhesives and then coated with filler material on rolling for uniformity. The major vegetable crop using seed coating / pelleting is lettuce in US. Brassicas, carrot, celery, onion, pepper and tomato are also coated / pelleted to a significant extent, varying with growing season, individual grower preference, the use of direct sowing or transplants, the economics of seed and coating costs etc.

Types of Coats :

Melt Coats

The melt coats dissolve when wet and gradually wash away from around the seed. The melt coats often require more water to wash the coating material away from the seed, and more time for the oxygen to reach the seed through the saturated coating material. Melt coats may offer advantages when soils are saturated.

Split Coats

Split coats initially retain their shape when wet and, by capillary action, pass moisture through the pill to be imbibed by the seed. The seed swells and cracks the pill by internal turgor pressure. The split coat often permits germination with less water, as they split, allow uniform, rapid oxygen access to the surface of the seed.

Materials used in pelleting

(i)Adhesives

Adhesives are gum arabic, methyl ethyl cellulose, gelatin, casein, carboxy methyl cellulose (CMS), methyl ethyl cellulose.

(ii) Coating materials or the filler materials

Lime, gypsum, dolomite or rock phosphate, charcoal materials commonly used for pelleting. Leaf powder of neem, arappu, pungam etc. are also used.

Advantages

- Pelleting regulates the size of seeds for precision planting.
- Reduces the amount of seeds required to plant the crop.
- Singling of seeds and prevention of clogging.
- Attraction of moisture.
- Supply or plant growth regulators and micronutrients.
- Saving of chemicals / fertilizers applied to the soil.
- Ensure uniform field establishment.
- Remedy for sowing at problematic soils.
- Protection from birds, animals and insects.

Disadvantages

- Seed pelleting increases the cost and weight of seeds.
- If proper pelleting techniques / machine is not used, there would be missing of seed during pelleting or some times a pellet may contain more than one seed.
- More moisture is required to germinate since pellet must be dissolved first.
- Delayed field emergence at low moisture level .

Polymer Coating

A thin polymer film smoothes the surface of the seed for better flow ability. The polymer also influences water uptake and the adherence of chemicals.

Traits of seed coating polymer

- Water-based polymer
- Low viscosity range
- High concentration of solids
- Adjustable hydrophilic/hydrophobic balance

- Capacity to form a hard film upon drying

Application of seed coating polymer

Similar to slurry seed treatments including the equipments to be used.

Advantage of film coating

- Minimal increase in seed weight allows the formulation to be changed several times during spraying and drying process so that the seeds can contain a multilayer film coat
- Absence of "dusting off" problem
- Better seed flow in planting equipment

Seed Extraction Techniques in Vegetable Crops

Dr. S. K. Goyal
Deptt. of Farm Engineering, Institute of Agricultural Sciences, BHU, RGSC, Barkachha, Mirzapur (U.P.)

The quality of vegetable crop seeds is not guaranteed at the grower's level due to a lack of information and expertise about the seed extraction procedure. Research on seeds has consistently sought for new technologies or even technological improvements to the seed production system, which has changed and improved the many stages of the seed production process. Any crop's seed technology involves a number of phases, including cultivation, harvesting, processing, drying, storing, transporting, and ultimately prompt marketing. Each step in the seed technology process is crucial to ensuring the quality of the seed. For vegetable crops, seed extraction is a crucial and initial step in the seed processing process. Vegetable crops' fully matured fruits are pulped by hand or machine, and the seeds are then separated from the inert material by mechanical means, washing, drying and winnowing, cleaning, packaging, etc.

Introduction

One of the most crucial activities to maintain the quality of seeds is to grow the crop for high-quality seed and to use the right seed extraction technique. The process of extracting seeds from fruit pods involves removing the seeds from the pulp, gelatinous material, chaff, straws, and other light components. Harvesting seeds with care will guarantee their great quality. Seeds should have the characteristics of the variety that was planted; for example, if a long, purple eggplant was planted, the fruit that was picked should have similar characteristics. The best time to harvest seeds is when the crops are at their fullest development. Overripe seeds should not be used since they may already be contaminated with pests and illnesses. Under-mature seeds won't grow healthy seedlings and typically won't even germinate. When a plant produces a large number of seeds, the seeds that will be used for planting are typically extracted or collected from the middle of the fruit, where the maturity of the seeds is ideal and they are all the same age. It is advised to purchase fruits that ripen in the midst of the fruiting season if early or late fruiting is not one of your selection criteria. techniques of seed extraction Fruit seed separation is a specialist task. A little carelessness when harvesting the seed might seriously impair its vigour and viability in addition to its outward look. Inadequate extraction methods can potentially cause in-situ germination.

Different methods of seed extraction

The selected well matures fruits of vegetable should be harvested for seed extraction. There are several methods of extracting from the vegetable fruit seeds namely:

- By rolling seeds in the hands
- By beating, walking, feeding, kneading for pulpy fruit like mango, mulberry, etc
- By spreading in the sunlight especially for cones, capsules, and pods which become open on sunlight.
- By winnowing to separate seeds from its husk and chaff by tossing it in the air or blowing air through it.
- By watering
- By floatation

  The seeds extraction from wet / flesh fruits can be done by the following methods.
  - Manual method

- Fermentation method
- Mechanical method
- Chemical method
- Juice and seed extraction method

A.MANUAL METHOD

(a). Crushing e.g., brinjal,
(b). Scraping e.g., cucumber
(c). Scooping e.g., pumpkins and
(d). Extraction e.g., squashes.

Dry Extraction

Dry extraction can be carried out manually or mechanically. Manual extraction involves hitting on a hard surface or using a flexible bamboo stick. To perform mechanical extraction, threshers are utilised. To prevent mechanical harm when using this procedure, caution must be exercised.

Wet Extraction

It is typically done with vegetables with fleshy fruits, such as tomatoes, brinjal, bittergourds, snakegourds, and ashgourds. Of them, brinjal and ashgourd extraction is simpler due to less obstruction from the meaty pulp. After being separated from the pulp, the seeds are washed in 0.1% HCI for two to three minutes to get rid of the sliminess. Curry powder grinder dry extraction of chilies is preferred to soaking in water and squeezing off the fruit rind. Either the fermentation method or the acid approach are used to extract tomato seeds. Although available, the citric acid procedure and the alkali approach (Na_2CO_3) are not frequently used.

The seed can be extracted/separated by following method:

B. CHEMICAL METHOD

(a). Alkali method-I: Fully ripened matured fruits are harvested and crushed to make pulp. In tomato, to hasten the fermentation process 0.5% sodium bicarbonate (500 g dissolved in 10 l of warm water) is added to the pulp and allowed to remain for a day. Then, the seeds are separated and washed free of alkali with water.

(b).Alkali method-II: 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.

(c). Citric acid method: In this method for seed extraction using 30 g of citric acid for one litre of pulp with digestion duration of 2 hour removes the gelatinous coating of seed without affecting the germination and vigour of seeds. But this method found to affect the storability of seed and used in only in tomato.

(c).Acid method

1. **Acid Method:** Large commercial seed producers frequently choose the acid approach because it results in exceptionally bright, clean seeds. The quickest way is another name for it. The fully grown fruits are collected and pulped using this technique. The pulp is taken in a plastic, wooden, or cement tub that is the right size. 30 ml of commercial HCL was added per litre of the seed and pulp mixture, and it was thoroughly mixed before being left for 30 minutes. During this time, the acid's corrosiveness dissolves the mucilage that was sticking to the seed and frees it from pulp. The seeds are then

properly washed 4-5 times with water to remove any remaining acid, as this will damage the seed embryo. Additionally, the seeds are brightly coloured, easily germinate, and fungus-free. Regardless of the variety, the various extraction techniques discovered that the acid procedure had a higher seed recovery %. With a 30-minute immersion in 2.5% HCL, germination was at its highest. The concentration of HCL varied amongst different veggies. some examples are given in the following table-1:

Table-1: Concentration of HCl for different vegetables for seed extraction

S. No.	Crop name	HCL concentration	Duration (Min)
1	Tomato	25 ml/1 kg of pulp	30
2	Brinjal	4 ml/1 kg of pulp	60
		10 ml/1 kg of pulp	45
		30 ml/1 kg of pulp	20
3	Pumpkin	1:6 (Acid: water ratio)	05
4	Cucumber	100 cc/1 kg of pulp	30

Seed extraction using acid method

The benefits of this method are:

- Seed extraction and drying is done on the same day,
- Higher seed recovery,
- The problems of low and high temperatures are avoided,
- Discoloured seed resulting from fermentation is entirely avoided and
- Remove external seed borne pathogens.

2. **Modified acid method:**Fruits that have just been harvested are pulped with water. The wet seed with mucilage is left after the peals and pulp have been removed. 1 kg of moist seed is produced from 10 kg of fruit. Then, 40 ml of commercial HCL is added, and the mixture is stirred continuously for 20 minutes to allow for reaction. After that, the seeds are cleaned and dried. With this technique, acid is conserved without compromising seed quality or seed recovery. This technique is typically applied to tomatoes.

C. FERMENTATION METHOD:

The fruits are mashed and let to ferment for two to three days in a non-metallic container. When temperatures are typical, it has been found that fruit fermentation takes place for two days, although this duration may change when temperatures are higher or lower. The seeds separate from the clinging pulp during fermentation and fall to the bottom of the container. The seeds are separated, properly cleaned, and dried in the shade until the necessary moisture content is reached. When compared to other methods of extraction, the seed recovery is lower. The pulp's fermentation and the fungal burden in the seeds both cause the seeds to lose their bright colour. Long-term fermentation may lead to in-situ germination. These techniques are applied to vegetables such as tomato, brinjal, cucumber, watermelon, and muskmelon, among others.

D. MECHANICAL SEED EXTRACTION:

Tomato, brinjal, and chilli are the main vegetables for which this technique is employed. The pulper machine in tomato is fed the known weight of matured tomato fruits. The pulp holding the seed is removed from the outlet separately, cleaned in water, and then dried in the shade. Pulper machines can also be used to crush the fruits of brinjal. A appropriate amount of water is added before employing pulpers, and the mixture is well agitated after pulping. When dried chilli fruits are put into the seed extractor's feed hopper, they are beaten in order to separate the seeds, which are then discharged out the outlet. The hulls were carefully removed from the seed. The efficiency of seed extraction was higher.

1. **Dry Method:**Harvested and dried in the sun for two to three days are the completely developed and dried fruits. After the seed is extracted, it is dried in the sun between the hours of 8 am and 11 am and 2 pm and 5 pm to restore the natural moisture content. Examples include chilies, okra, sponge and ridge gourds, among others.
2. **Directly harvesting of matured pod method:**The pods are harvested when they are fully developed and then sun-dried for a couple of days to get the moisture content down to 15%. The seeds are then extracted by beating the pods with a flexible bamboo stick. To lessen mechanical harm to the seed, excessive drying and beating should be avoided. Seed quality declines as a result of severe mechanical damage. Okra, French beans, Lablab beans, cowpeas, cluster beans, etc. are the principal crops that use this technique.
3. **Manually seed extraction method:**when the fruits are hand seeded after being chopped into longitudinal pieces. The seed is dried and the pulp residue is cleaned off. This process significantly affects seed storage ability as well. Examples include bitter gourd, pumpkin,etc.

Cleaning of seed:

Some detritus, such as pieces of cones, capsules, pods, etc., are also with the seed after the seed has been removed from the fruit. Additionally, there are certain seeds that are ill. Debris must therefore be removed because failing to do so will hinder germination.

Pre-cleaning:

The condition of the gathered seed must be assessed after collecting and before storage. It becomes quicker in the case of pods. In order to prevent further fermentation, moist and fomenting pods must be spread out to dry and treated with a fungicide.

Methods of cleaning:

a. **Water method:** Put the seeds along with debris in the water. Seeds will settle down and debris will start floating on the surface. Remove the debris and let the dry.
b. **Winnowing method:** Put the seeds along debris in the winnowing apparatus. Drop the material from some height in the direction of the wind as a result seeds will fall down on the ground and debris will blow off.
c. **Hand picking:**If the debris which is mixed with seeds is easily removable then clean the seeds with hands.
d. **Sieving method:** This method is applicable when the amount and size of debris are smaller than seed. In this method, seeds along with the debris are put in sieves and then by shaking, the debris pass through sieves and seeds remain in the site.

Seed Extraction of Some Vegetable:

Chilli: Red-ripe fruit is selected, sun-dried, then mechanically macerated to separate the seeds. Early fruit harvesting will have an impact on germination. With the help of a flexible bamboo stick or a chilli seed extractor, dried fruits are placed in a gunny or cloth bag and threshed.

Tomato: Fruits that have reached maturity are crushed to create pulp. 25 to 30 ml of commercial HCl acid should be added for every 1 kg of pulp, and the mixture should be constantly stirred for 30 minutes. The seeds are rinsed three to four times in water after 30 minutes, then gently dried in the shade for one day to get the moisture level down to 7-8%.

Cauliflower: When the pods become brown, the early grown plants are often picked first. Harvesting can be done in two batches. After harvesting, it is piled up for 4 to 5 days of curing before being turned upside down for an additional 4 to 5 days of cure. The pods are then moved using hand sifters and threshed with flexible sticks.

Gardenpea: The entire plant is uprooted and gathered on the threshing floor after roughly 90% of the plant's pods mature and turn dry. The seeds are removed from the pods by threshing and winnowing after about a week. A thresher performs threshing, and utmost caution must be used to avoid damaging the seed. The dried, ripe pods can also be picked up by hand and lightly threshed. The moisture content of seeds is typically higher during this period, hence drying must be used to keep the seeds at the required 8% for regular packaging and 6% for vapour proof packaging.

Okra: The okra seed pods must dry on the plant and start to split or crack before they can be harvested. Late-maturing fruits have immature, lighter seeds that grow into weaker seedlings. They are also linked to seed infections, which lower the fruit's total germinability. Fruit placement and size affect assimilate conversion and seed fullness. The middle node bold seed has the greatest vigour and storage capacity. If fruit position considerations are taken into account, good quality seed with sufficient germination potential could be produced under favourable field and weather circumstances.

Ashgourd: Fruits should first be sliced in half lengthwise and crosswise. After that, extract the seed and pulp and smash them by hand in a lot of water. Eliminate the floating portion, then gather the seeds that have sunk to the bottom. Acid extraction of the seed is another option. Take the pulp and seeds, add HCl that has been diluted six times with water, and mix for 30 minutes. The floating portion must be removed as the seeds settle. Gather the seeds that have fallen to the bottom and wash them three to four times in water. The seeds obtained by the acid process may be dried quickly and the fungal development on the seed coat can be easily removed, giving the seeds a bright golden yellow colour and good vigour. Due to fungal activity, the seed extracted using the fermentation method may be weak and have an unattractive colour.

Chaudhary (1957) has also given the following methods for seed extraction from slimy mass of Tomato:

1. **Fermentation method:** The seeds from the fruits chosen for this procedure are completely lost. The chosen ripe fruits are collected and stored in wooden crates for a day. They are manually smashed; no fruit juice should flow out. Depending on the high and low temperatures, the entire mass is stored for 24 to 72 hours. Fruit liquid will then separate into seed and flesh, which will float to the top. The seeds are rinsed 8–10 times with clean water to eliminate the fermenting bulk, after which they are dried. A protracted fermentation could harm the seed.
2. **Alkali treatment method:** Selected fruits are split in half, and a slimy mass containing the seeds is scooped out and placed in a container with a wooden stick. The mixture is then treated with 300 g of washing soda in an equal volume of 4 litres of boiling water. After cooling, the mixture is left to stand overnight. The entire seed settles to the bottom the following day. The seeds are properly cleaned.
3. **Quickest method or acid treatment method:** Using commercial hydrochloric acid at a rate of 75 ml per 12 kg of material, slimy mass is separated and processed. The fast

dispersion of colloidal material from seed by acid is what causes seed separation. The seed is freed from sliminess in 15 to 30 minutes thanks to fermentation, a microbial process, rather than fermentation. After decanting out the acidified liquid, the seeds are thoroughly washed with water.

EXTRACTION / SEPARATION OF VEGETABLE SEEDS

If you have a mixture of vegetable seeds, different procedures are used to separate the seeds from the mixture's contaminants as mentioned below:

A. Separation based on weight (or specific gravity)

Cleaning seed by differences in specific gravity is one of the oldest seed cleaning techniques. Winnowing is the term used to describe manual wind-driven work. On the most basic level, materials and seed are thrown in front of a wind source (either natural wind or a fan). Lighter materials are carried further away from the wind source while heavier things fall closer to it. This is a very effective way to swiftly clean seed on a small-to-medium scale. A fan is commonly found on screen cleaners to help blow off some dust and chaff. The machines that are utilised the most frequently are gravity tables. By varying seed weights, they may separate seeds. In order to operate, gravity tables blow air up through the body of the device, which supports a fine-screened, slanted "deck" on the top. A hopper supplies the seed, which travels across the deck. The screening material has a little nap that "holds" the seed because of the narrow screen apertures, which prevent any seed or material from passing through. Lighter material is lifted by air passing through the screening and subsequently "floats" down the deck while heavier material is kept in touch with the screening material due to the nap and moves up the deck.

B. Cleaning wet-seeded crops

The seeds that are formed in fruits like tomatoes, cucumbers, and squash are known as wet seeds. The fruits are crushed by hand or with a wet seed extractor during processing. Following the extraction, a fermentation process is advantageous for several wet-seeded seed crops. Others only need to be thoroughly cleaned and dried.

a. Wet processing of seed: It involves following three steps:

1. **Removal:** Wait for the fruit to fully develop on the plant before harvesting any seeds found inside fleshy fruits and vegetables such as squash, cucumbers, and tomatoes. Fruit is typically collected at a stage that is considerably more advanced than the stage at which the fruit would be harvested for consumption. After cleaning the fruit, remove the seeds by breaking it open. Wash or ferment the seed to remove pulp residue.
2. **Drying:** Spread the seeds out on a tray in a warm, dry place, and let dry. Seeds should not reach temperatures over 35^{o} C.
3. **Fermentation:** In order to remove the pulp from seeds like tomato, pepper, cucumber, and squash, as well as in some cases to eradicate specific bacterial seed-borne diseases, fermentation is often used in seed processing.

b. Fermentation of wet-seeded crops: It involves following nine steps:

1. Rinse dirt and debris from fruit.
2. Mash the fruit and pour the mixture of seeds, pulp, and juice into a large container (garbage cans work well).
3. Place bucket in a location at 24-27^{o} C. Ferment tomatoes for up to three days and squash for up to 2 days, depending on the ambient temperature. At 24-27^{o} C, fermentation may require 42 to 72 hours.
4. Stir the fermenting mixture two or three times a day to aerate the mixture and facilitate even fermentation.
5. In two to three days a white scum may appear on the top of the mixture. This is normal and indicates that fermentation is successfully taking place. After the two- to three-day

period, seed is ready to be rinsed. Pour off the top layer of scum and pulp. Pour water into the remaining mixture so that the volume is doubled.

6. Stir, allow the mix to settle again, and pour the top layer of pulp and debris off the top. Some lighter, less viable, seed may be poured off with this top layer.
7. Repeat this washing process from 3 to 6 times, until the water is fairly clear.
8. Pour the remaining contents (seed) through a large strainer retaining the seed and draining off the remaining water.
9. On a fine screen, spread out the drained seed to dry. For the majority of seeds, fine mesh window screening works nicely. Spread out the seed on the screen to get the smallest possible seed layer (less than 0.25 inch). Avoid putting seed on paper since the paper may retain moisture and dry seed may attach to it. To ensure an equal drying process, stir the seed frequently. To speed up drying, if at all feasible, position the seed in front of a fan or light breeze.

Curing of the vegetable seed

To prevent the branches from drying out quickly during curing, the harvested crop is heaped up in small piles and covered with hay or a tarpaulin. Curing can take place on a cement floor, tarpaulin, or both. Curing with branches slows down the ripening process, enhances the colour of the seed, and lowers field losses due to shattering. The heap is turned upside down and given another 4-5 days to cure after the first 4-5 days. If harvesting is done during a wet, humid season, caution should be used, and the stock should not be stored in this condition for longer than 4-5 days. In cauliflower and cabbage, this technique is employed. To shield heaps from direct sunshine, paddy or wheat straw is used as a covering. The mounds need to have tarpaulins in case of rain. In comparison to sandy loam, heavy treatment soil may need more time to cure.

Drying of the vegetable seeds

Natural moisture present in seeds at harvest time is frequently higher than that needed for the optimal germination and longest possible life. The seed's moisture content is likely the most significant element affecting its lifetime and ability to germinate. The moisture content of the seed after it has been separated from the mother plant depends on the relative humidity and is equal to that of the air around it. Fruits with fleshy seeds, like tomatoes, cucumbers, and others, may absorb more water during the wet extraction process due to their significantly greater moisture content upon harvest. On the other hand, seeds developed in fruits, such as those of onions, amaranthus, brassicas, etc., that become dehydrated during the ripening process, are rather dry at the time of harvest. Freshly harvested vegetable and flower seeds in humid tropical environments may have a moisture content of 18 to 35%, which must be decreased to a "safe level," or roughly 8% for regular packing and 6% for vacuum packing.

Packaging of the vegetable seeds

To preserve the seeds' ability to thrive, it is crucial to use the proper material and packing method. Since this is the location where your company's profits and losses are determined, incurring extra costs here is by no means a problem. It is a significant loss if the seeds are improperly packaged and spoil even before they reach the customers. Or perhaps the seeds were delivered to the clients but did not germinate because the packaging material was toxic. If your business has a bad image, clients will naturally choose one of your industry's rival sellers as their next course of action. Paper is the ideal material to utilise for packaging. Modify the paper however you like to store your seeds, but avoid sealing it fully to prevent the seeds from being active.

Conclusion

The development and distribution of high-quality seed would be the most crucial and practical strategy to increase the yield of vegetable crops. It is difficult to overstate the significance of high-quality seed because it is essential for high output. Extraction is the process of selectively removing a compound of interest from a mixture using different techniques. Choosing the best technique is depends on various conditions. A suitable method for any vegetable is a very important task because the recovery and quality of seeds are depends on it.

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Post-Harvest Management for Seed Quality Assurance in VegetableCrops

Dr. S. K. Goyal
Deptt. of Farm Engineering, Institute of Agricultural Sciences, BHU, RGSC, Barkachha, Mirzapur (U.P.)

Abstract

'All the flowers of all the tomorrows are in the seeds of today' - Swedish proverb

To address the difficulties of an ever-growing population and food security, quality seeds are unavoidable. Due to its role as a technological carrier, seed throughout time changed into a commodity used in commerce. There is potential to increase earnings through the export of vegetable seeds because India is the fifth-largest player in the global seed market and a wide variety of vegetable crops seed are produced under various agro-climatic conditions. The International Seed Testing Association (ISTA) methodology of seed testing and the Organization for Economic Cooperation and Development (OECD) standards govern the global seed quality assurance system for export. In India, seed quality assurance is governed by the Seeds Act 1966, which stipulates that quality seed must meet the requirements of the Indian Minimum Seed Certification Standards (IMSCS). For Indian seeds to carve out a unique place in the global seed market and to facilitate international seed trade, it is necessary to align the country's current quality assurance system with OECD and ISTA standards.

History and development of Indianvegetable crops seed industry

India is the world's second-largest producer of vegetables, with an area of 6.2 million hectares and an average productivity of 15 tonnes of fresh vegetables per hectare. However, this production is insufficient to feed our expanding population. Due to a lack of high-quality vegetable seeds, low vegetable yield was noted. Since ancient times, Indian farmers have relied heavily on regional varieties and farm-saved seeds, whose quality cannot be guaranteed. This has had a significant negative impact on vegetable production. Following Independence, the Indian government gave more consideration to the creation of seed programmes in all of its five-year and annual plans. The creation of High yielding varieties has advanced significantly since the foundation of AICRP (vegetables). It was totally reliant on the reproduction and dissemination of seeds from the recently created types. Originally, public sector organisations like NSC, SFCI, SSCs, SAUs, ICAR institutes, etc. produced vegetable seeds. However, today, their share is minimal, and the majority of the nation's need for vegetable seeds is still satisfied exclusively by private seed enterprises. Still, open pollinated varieties make up the majority of projects in the public sector. The productivity and overall production of different vegetable crops have increased dramatically with the use of high-quality seeds of enhanced varieties or hybrids.

Problems in vegetable seed industry

The unorganised seed sector, where seed traders directly buy from growers and distribute under many trade names, handles a sizable portion of the vegetable seed business. Only a few reputable and well-established seed businesses have internal R&D programmes for seed quality assurance and crop improvement. These businesses sell seeds in India that they both generate themselves and import from their partners abroad. The private sector has little control over the production and selling of vegetable seeds, in part due to the proliferation of local small seed businesses and the number of seed traders. There is a trend toward the development of hybrids and hybrid seeds as a result of the constant demand for high-quality

seed to enhance vegetable production. Vegetable hybrids have just recently been created and released by the public and private sectors. Most of the current, potential hybrids come from the private sector. There are now 54 public sector vegetable hybrids available. The creation of potential F1 hybrids has greatly benefited from the efforts of a number of private seed firms, including Namdari Seeds, Syngenta, Bejo Sheetal, Mahyco, Century, Ankur, Indo American Hybrid Seed Company, etc.Despite the cost, private seed firms' hybrid seeds are the most widely used and preferred by vegetable growers because to their guaranteed supply and increased output. Due to the favourable weather conditions for the production of quality F1 hybrid seeds of tomato, brinjal, chilli, cauliflower, cabbage, okra, melons, cucumber, and gourds, the majority of private seed companies are concentrated in southern India, particularly Karnataka, Maharashtra, and Andhra Pradesh. The states of Himachal Pradesh and Jammu and Kashmir generate all F1 hybrid seeds of temperate vegetables, including late cauliflower, cabbage, garden beet, temperate carrot, radish, and turnip.

Scope for vegetable seed production in India

Only behind China, India is the world's second-largest producer of vegetables. In India, 6.2 million acres of land are used to grow vegetables, yielding 94 mt. By 2025, our nation's vegetable consumption is anticipated to reach 235 mt. Vegetable crop productivity and quality can be significantly increased by using high-quality seed, fertilisers, irrigation, plant protection techniques, and other appropriate agronomic practises. Among these, using high-quality seed is crucial. Economically speaking, the cost of seed is lower, but it is only recognised when good quality characters are present. The significance of seed quality is emphasised since low-quality seeds "may turn into seeds of frustration." It is crucial to use seed that meets the required standards for excellent genetic and physical purity, physiological quality, and health. The majority of public sectors place a high priority on open pollinated cultivars, of which wheat and rice make up roughly 60%.Due to their focus on high-quality, low-volume seeds, the private sector is where the majority of promising vegetable hybrids come from. Since a lot of land is still being sown using farm-saved seeds, private businesses have a good scope and opportunity to sell seeds. About 67% of seeds are produced by private seed businesses, and farmers store their own seed. Almost 33% of the entire seed requirement is provided by government organisations, including public sector businesses at the central and state levels. Though the predicted demand for vegetable seeds is currently 200 t for temperate varieties and 20,000 t for tropical and subtropical ones, it is expected to rise steadily over the coming years. Garden pea makes up between 6,000 and 7,000 tonnes (t) of the vegetables, bhendi about 5,000 tonnes, and onion 2,500 tonnes.

Factors affecting quality vegetables seed production

In the location where they are widely used, cautious seed production of vegetable varieties and hybrids should be done. Climate variables directly affect the production of high-quality seeds. They are light (duration, intensity, and photoperiod), low, moderate, high, and very high temperatures, as well as total and distributional rainfall and snowfall and wind (direction and velocity). The most crucial element is the temperature, and dry temperate climates are typically best for seed production. For instance, only dry temperate regions that meet the chilling conditions can produce cabbage seeds. Vegetables can be divided into temperate and tropical kinds and require varied climates for successful seed production. The regular onion bulb crop may bolt more easily due to the climate. Additionally, photoperiod has an impact on onion seed production and bulb yield. The different elements influencing the production of high-quality seeds are:

1. Agro-climatic factors / Ecological factors – edaphic and climatic factors
2. Production factors

3. Post harvesting handling of seed
4. Seed quality control factors

Post-harvest handling of vegetable seed crops

1. Harvesting and threshing: The harvests for veggies must be taken in several pickings. The first and last one or two pickings can be used for veggies to increase production. This can encourage the plant to grow even more so that it can produce more fruit or pods. The seed recovery and quality in the aforementioned pickings will also be subpar. The seeds from the harvested produce should be extracted using the appropriate extraction techniques, such as dry extraction for bhendi, chillies, crucifers, ribbed gourds, peas, and beans, and wet extraction for tomatoes, brinjal, bitter gourds, snake gourds, and brinjal.

2. Drying and grading: Direct sunlight exposure can degrade the quality of seeds, thus if necessary, seeds can be dried in a shelter with multiple openings under diffused sunlight. Blow dry air at a temperature between 21 and 30 ^{o}C, never more than 43.5^{o}C. Different grader types are used for grading.

3. Seed treatment: Fungicides and insecticides are applied to seeds to prevent the spread of diseases and insects, as well as their new entry into the seed.

4. Packaging, labelling and sealing: It is important to use the suitable containers with the proper labelling and sealing (seed moisture content at proper level).

5. Movement and storage: Precautions should be taken to avoid seed deterioration while in transit and or in storage. Ideal storage for long term is dry and cool condition. Ideal condition can be maintained if the sum of RH and Temperature does not exceed 38 ^{o}C.

Vegetable crops seed quality control factors

1. Seed moisture: The seed moisture affects seed storability. Seeds with low moisture store longer and remain free from insect pests.

2. Germination per cent: To produce crops for the coming crop cycle, seeds are sowed. Thus, the percentage of seeds that germinate shows if seeds have the capacity to grow and establish into seedlings in the nursery bed (open or polythene) or in the main field. Germination percent is the measurement for this quality. Pure Live Seed (PLS), which has superior viability, is a blend of 100% pure seed and 100% germination.

3. Vigour: It shows that a seed can develop in a variety of settings or microclimates in the fields where it is planted. High seed vigour is frequently associated with high germination percentage, though this is not always the case.

4. Storage life: The most significant element impacting viability loss during storage is seed moisture content. Most expensive vegetable seeds are packed in attractive, moisture- and vapour-proof containers, so they are not damaged during storage or transportation. However, some large-sized seeds, such as garden peas and beans, are packed in porous containers, so their moisture content varies depending on the relative humidity of the air.

5. Seed health: Neither seed-borne illnesses nor insect infestation should exist in vegetable seed. Embryos are typically destroyed by insect infection, rendering seeds unsuited for planting. Similar to this, most bacterial and viral illnesses are transmitted through seeds. In addition to contaminating the crop, they hasten the spread of illness. To avoid contamination and spread, seeds must be free of pests and diseases and treated with a pesticide or fungicide.

6. Mechanism of control: Inspections, sample testing, and the enforcement of minimum criteria are all part of the widely accepted system of seed certification. These elements together provide the mechanism for seed quality control.

Physiological and harvestable maturity for vegetable crop seeds:

Seeds are ready for harvest after the attainment of physiological maturation.

1. Physiological maturation: It is the phase where the seeds have accumulated the most dry materials. The maximum dry weight of the seed, germination potential, and vigour potential are stated with the seed's moisture content at this stage, which will be in decreasing order (25-30%). Due to variable flowering habits, the physiological maturity is depicted for each individual seed but will differ for the population.

2. Harvestable maturation: The harvestable maturity stage is defined as the point at which 80% of the population has reached physiological maturity. At this stage, the moisture content of the seed will be lower than it was during the physiological maturation stage (18–20%). In order to obtain high-quality seed, the seed crop is typically harvested at the point of harvestable maturity.

Harvesting

The time of sowing should be adjusted in such a way that the maturation does not coincide with rainy season or at high humidity weather periods. Points to be kept in mind for harvesting the vegetable seed crops as given here under:

- The moisture content.
- Physiological maturity.
- Avoid delayed or premature harvest.
- Once over harvest in agricultural crops.
- Staggered harvest in the horticultural crops.
- Harvesting symptoms according to the different crops.

In general, the crop that is harvested when it is ready to be harvested will produce more seeds. Based on the anthesis and fertilisation times, crop maturation is not necessarily uniform; instead, there will be a mix of matured, immature, and over matured plants. As a result, there must be an ideal time to harvest a certain seed crop since after that point, losses will outweigh the potential seed output. Natural seed drying on the plant is substantially accelerated by hot, dry weather. The most significant indicator of a crop's readiness for harvest may be the seed moisture content. Depending on the condition of the seed upon harvest, vegetable seed crops are categorised into three categories.

a. Dry seed: The seed is usually dried on the plant before harvesting e.g. okra, brassicas, lettuce, peas, beans, beet and onion.

b. Fleshy fruits: Ripe fruits are removed from the plants and first dried. The dried fruits are afterwards opened to remove the dried seeds, such as those from bottle gourds, ribbed gourds, and chilies. c. ripe, moist fruits The seed of fruits with a lot of moisture adheres to a gelatinous or mucilaginous coating. After seed extraction, this needs to be eliminated through a fermentation process or a treatment with weak acids. When such fruits reach maturity and ripeness, they are harvested. (For instance, pumpkins, cucumbers, and brinjal).

Harvestingmethods: Depending on the volume of production, the cost and the accessibility of experienced labour and/or adequate harvesting machinery, seeds and fruits are either manually or mechanically harvested.

1. Hand picking: Picking fruits by hand is a convenient way to collect the seeds of some crops, such as solanaceous fruits (brinjal, pepper, tomato), cucurbits, and sweet corn. A knife or scissors can be used to chop the little seeded fruits or seed heads of vegetable crops like onion, carrot, okra, or chilli. Often, as with lettuce, chicory, brassicas, radish, and peas, it is advisable to use a matchet or sickle to chop off the entire plant. However, to harvest the legumes, the entire plant is typically pulled up, and the plant is then threshed to extract the seed (e.g. peas and beans). Despite being labor-intensive, hand harvesting techniques allow for the picking of individual plants or even crops at different stages of development. Compared to mechanised harvesting, manual harvesting offers greater protection and the highest possible seed

production per unit area. The greater the plant component that is cut and removed together with the ripening seed produces a better seed production in plants that require post ripening (e.g. the small seeded vegetable crops like lettuce and brassicas).

2. Mechanical harvesting:A appropriate mechanical harvester can be used to harvest vegetable seed crops, especially on large-scale commercial seed farms when manual labour is expensive. Cutting and threshing tasks can be completed by two different machines during mechanical harvesting, or both tasks can be completed by a single combined equipment. Mowing windrowing devices, which are especially useful for crops like peas, beans, spinach, carrots, and brassicas, can automate the cutting activities.

Threshingand seed extraction methods:

Carefully harvested produces are to be carefully threshed / extracted to remove the seeds from fruit / panicle / pod. Removal of seeds from dry fruits is known as threshing while that of wet fruits is known as extraction

A. Threshing / Extraction: Threshing involves beating or rubbing the plant material to detach the seed from its pod or fruit. The detached seed is then winnowed to remove chaff, straw and other light material from the seed.

Traditional threshing methods: Seed has to be extracted from dry seed heads (e.g. onion, lettuce, brassicas), dried fruits (chilli, pepper and gourds) or from fleshy fruits like tomato, cucumbers and melons in which the seeds are wet at the time of extraction. Threshing may be carried out by flailing, beating or rolling the seed containing material to separate it from other plant debris or straw. It may be performed manually, with animals or mechanically. Hand threshing is simplest and can be a cheaper method if sufficient labour is available. Seeds may be hand rubbed, beaten against a solid wall or on the ground with sickel or flail. Thickness or depth or the plant material being threshed should be sufficient to avoid damage to the seeds.

1.Mechanical threshing: 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.

2. Hand threshing for 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. e.g. Sweet corn.

d. Rolling – seed materials is rolled on threshing floor or tarpaulin repeatedly and seeds are easily separated.

e. Walked on – the seed material is spread on the threshing floor and children or other persons are asked to walk on the seeds materials till the seeds are separated. Seeds which have been hand threshed are usually still mixed with the plant debris and further separation is done by winnowing or sieving.

Seed extraction from wet or flashy fruits: The selected fruits are harvested for seed in the same way that is picked for the market. The seeds extraction from wet / flash fruits can be done by the following methods.

1. Manual method
2. Fermentation method
3. Mechanical method
4. Chemical method
5. Juice and seed extraction method

Grading: The threshed produces are precleaned either manually or mechanically and are graded using different but optimum sieve of specified sizes. This grading brings homogeneity in the lot which aids in obtaining uniformity among the population in the subsequent sowing.

Drying methods for vegetable seeds:

Seed drying means removal or elimination of moisture from the seed to the required level is called drying. Drying of seeds is done by following methods:

1. Sun drying(Natural Drying)
2. Forced air drying (Mechanical drying)
3. Use of desiccants (Chemical) for drying

1. Natural Drying (or) Sun drying: Here the seeds are uniformly spread over clean dried yard and allowed for drying to the required moisture level. The seeds should not be dried under hot sun during 12.00 noon to 2.00 pm as it causes damage to seeds by UV rays. This method depends on weather conditions, which are unpredictable one.

Advantages:

- Easy process
- Cheap method
- Requires no additional equipment
- Does not require any expenditure on electricity or fuel Disadvantages
- More chance for mechanical admixture
- Seed loss is more while drying due to insects, birds and animals.
- Takes long time for drying.
- Uneven drying.
- High weather risk and damage due to sudden rain or heavy wind.

2. Mechanical drying/ artificial drying

Forced air is used for seed drying by the following three means.

a. Natural air drying: Natural air is blown upon the seeds using suitable air blower for drying. Continuous drying is possible in this method. In modern seed godowns provisions are made to forcible circulation of air with the help of electric blower or fan. If the outer air is comparatively dry, this method is followed. So it is possible only during dry months.

b. Drying with supplemental heat: Small quantity of heat is applied to raise the air temperature to 10-200 F for reducing the relative humidity of air used for drying. In this, drying is performed quickly due to use of dry air, but continuous drying for long period affects seed quality.

c. Heated air Drying: The air is heated considerably as much as by 40 ^{o}C and used for drying the seeds. Very quickly the seeds get dried. The seeds should not be continuously dried as it causes damage to seed. High moisture seeds should be dried by this method.

Advantages

- Quick method
- Perfect drying is possible even under unfavourable weather condition.
- Seed loss is minimized.

Disadvantages

- Requires specialized equipment and machine, which is costly.
- Care should be taken while drying the seed using hot air, as it causes damage to the seed.
- Tempering is to be followed while drying the seed in this method.

Types of driers

1. Metal bin drier
2. Vegetable seed drier
3. Batch drier

Metal bin drier: Here the seeds are placed in a metal bin and the heated air is blown in to the bin through the perforations made at the bottom of the bin. In this uniform drying of all layer is not possible for which decide the thickness of the seed layer to be taken to the bin and also have to stir the seed manually or mechanically at regular intervals.

Vegetable seed drier: In this drier, the seeds are separated over the bottom screen seed trays which are kept inside chamber or cabin. The heated air is passed to dry the seed. The heat is generated by electrical source and the air is passed through trays. Here uniform drying is possible.

Batch-Drier: In bin batch dryers, the seed is placed in a (usually round) bin, and ambient or slightly heated air is blown through it by a fan. The maximum thickness of the seed layer in the bin depends on the initial moisture content, the type of seed, the air temperature and RH and fan horse power. To obtain a uniform airflow through the seeds, a full perforated floor is required. A layer of seed 0.8 to1.0 m at 20% moisture can be dried to 14% within 24 hrs without affecting germination at 30-35^{o}C and 50-60%RH air at a rate of 5-8m^{3} per minute per m^{3} of seed. After the seed in a bin has reached the acceptable average moisture content, a moisture gradient will remain from the top to the bottom of the seed. The surface layer will have a moisture content above the average and the bottom layer of the bin will be lower than average. Thus, proper mixing of the seeds is essential before further storage or packaging. This can be addressed by installing one or more grain stirrers to mix the entire content of a bin for 3-12 hours.

Wagon Batch- Dryer A seed transport wagon can be transformed into a wagon batch-dryer by equipping it with a plenum, a perforated floor, and a fan/heater unit coupled with a canvas transition to the wagon. The drying principles of a wagon batch dryer and a bin batch dryer are similar. Wagon batch dryers are most frequently used for drying fragile seeds such as large-seeded legumes (eg. field or garden beans and peanuts). The recommended air flow rate for the ambient –air wagon drying of a 1.5m layer of peanut seeds is 0.25m3 of air per m2 of floor area.

Use of seed desiccants (Chemical drying):

In this technique, the moisture from the seed and its surroundings is absorbed using silica gel or fused calcium chloride ($CaCl_2$). Silica gel comes in two varieties: indicator type and non-indicator type. Lithium chloride, the active element in silica gel, is what causes drying. Up to 15% of its weight in moisture can be absorbed by silica gel. Therefore, we can use this to get very low moisture content, which is not achievable with mechanical driers. - The indicator type will be blue in colour and turn pink after absorbing moisture. So, after dehydration, we may extract this and reuse it. Non-indicator types are always white and maintain their colour even after absorbing moisture. Therefore, this kind does not indicate anything. However, following dehydration, this can also be employed again. - The majority of the vegetable and flower seeds used as breeding material include calcium chloride. Here, a greater quantity is required. Ten percent of its weight can be absorbed. - The technique is only appropriate for drying a few seeds at a time. It is a more expensive and complicated approach.

Advantages

- Less time consuming
- Drying rate is uniform

Disadvantages

- It cannot be used in large scale
- A skilled person is required to monitor the operation

The rate ofvegetable seed drying depends upon the following factors:

1. The moisture content of the seed
2. The existing relative humidity and temperature of the environment
3. Depth of spread of seeds
4. Rate of air blow
5. Drying temperature
6. Size and capacity of the drier and
7. Kind of seeds.

Tempering:When warm air is used to dry a seed, the moisture content in the seed's outer layer is eliminated more quickly, while the moisture inside the seed tries to diffuse slowly outward to maintain balance. Due to the difference in moisture content between the seed's dried exterior layer and its inner layer that is drying during continuous drying, a pressure gradient forms inside the seed. As a result, hair-like fissures start to form inside the seed, which damages the seed. Therefore, tempering should be used. It refers to stopping the drying process for a certain amount of time to allow the moisture in the seed's inside to move evenly across the external section.

Seed processing

A crucial component of the overall technology used to make high grade seed accessible is seed processing. It guarantees high-quality seeds with little adulteration to the final consumers. The word "seed processing" in agriculture refers to cleaning, drying, treating the seeds, packaging, and storing them. The term "seed processing" can be used to describe all post-harvest activities aimed at enhancing the viability, vigour, and health of seeds.

Purposes of seed processing

1. To reduce the price of other processes, such as storage and transportation. This is accomplished by clearing debris from the area around the seed lot and removing any empty or broken seed (pre cleaning).
2. To increase the longevity of seeds; by drying seeds to safe moisture content and treating with protective chemicals.
3. To reduce the variability in vigour by invigorating the seeds and removing the low vigour seeds.
4. To improve the uniformity in seed shape or size by grading or by pelleting.

Principles and objectives: The quality of seed is improved during processing in two ways:

1. Separation of other seeds or inert matter and
2. Upgrading or the elimination of poor-quality seeds.

Getting the most pure seed with the greatest chance of germinating is the ultimate goal of seed processing. Heterogeneity characterises the threshed produce. Produce becomes homogeneous thanks to processing. This homogeneity aids in achieving field uniformity. In seed processing facilities that have been approved (by the director of seed certification), seeds are processed. The goal of seed processing is to reduce the degree of heterogeneity in the seed lot by employing the best processing techniques. The heterogeneity occurs in a seed lot due to following reasons:

Causes for heterogeneity:

- Variability in soil for fertility, physical, chemical and biological properties
- Variability in management practices (irrigation, application of nutrients etc.)
- Variability in ability of the seedling for utilizing the inputs
- Variability in pest and disease infestation
- Position of pod or fruit in a plant or the position of seed in a pod.

This heterogeneity can be narrowed down in the processing of seeds by eliminating the undersized, shriveled, immature, ill filled seeds using appropriate sieve size. The germinability and vigour of the seed lot can be upgraded by grading the seeds according to size, specific gravity, length and density of the seeds. The inherent qualities such as germinability and vigour are exemplified by certain physical characteristics of the seed i.e., large size, a denser seed, optimum length etc., So, if grading is done to obtain a particular range of size, shape, length and density of the seeds, the quality of the lot is upgraded.

Requirement for vegetables seed processing

- There should be complete separation.
- There should be minimum seed loss.
- Upgrading should be possible for any particular quality.
- There should be have more efficiency.
- It should have only minimum requirement.

Types of materials removed during vegetables seed processing

- Inert materials
- Common weed seeds
- Noxious weed seeds
- Deteriorated seeds
- Damaged seeds
- Other crop seeds
- Other variety seeds
- Off-size seeds.

Operation sequence in seed processing

Operation sequences are based on characteristics of seed such as shape, size, weight, length, surface structure, colour and moisture content. Because each crop seed possesses individually seed structure. Therefore, sequence of operation will be applied proper equipments. However, sequences of operation in seed processing are drying, receiving, pre-cleaning, conditioning, cleaning, separating or upgrading, treating (Drying), weighting, bagging and storage or shipping.

Grading and upgrading equipments

Sound seeds may be distinguished from inert matter, sterile and empty seeds by size and shape, specific gravity, colour and surface texture. Processing has well been mechanized and based on the physical characters of seed, separate machines are available for grading and upgrading, and these machines can be used either singly or in combination.

Packaging and storage

When the seeds are already dried and cleaned, they have to be stored properly before planting. The seeds are placed in an appropriate package or container and stored until planting. They should be packed and stored properly to maintain high viability until planting time. Seed moisture content and temperature are the two important factors that affect seed viability in storage. When these factors are too high, the seeds deteriorate rapidly.

Packaging

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 of the air surrounding it. 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 to moisture. This is significant if one takes into account the general rule for predicting storage life of seeds which states that seed life is doubled or halved with every 1% decrease or increase of seed moisture content. This rule applies to storage between 15-70% RH, 0-30^{o}C, and 4-14% moisture content. The nature of the packaging material, therefore, is very important in this regard.

The temperature of the seeds to be packed is one factor to consider. Warm seeds heat up the surrounding air and increase the capacity of the air to hold water vapor. If the seeds are tightly sealed inside and then cooled down, the excess water vapor in the sealed container condenses; and a significant portion of this is reabsorbed by the seeds. This in turn increases the seed moisture content of the previously dried seed. In serious situations, the seed may become wet enough for fungi and insect pests to grow. This is especially critical if the ambient relative humidity is high, and the air space in the container is much larger than that occupied by the seeds (i.e., too big container for too little seed). It is, therefore, advisable to choose a container which can be packed as full as possible with the amount of seeds available. Apart from packaging materials, the packaging procedure is also very important. Some of the most suitable and readily available seed containers for vegetable gardeners are small glass bottles with a good rubber-gasket screw top, e.g., baby food, soft drink, soya sauce, and wine bottles. The bottle stoppers should be sealed with molten wax or candle after being tightly secured. Small tin cans with air-tight lid, (tea or coffee cans, health drink containers, etc.) may be used. It may also be useful to wedge a layer of plastic sheet between the lid and the container. The seeds may also be kept inside a thick polyethylene (PE) packet which is fastened or sealed as tightly as possible before being packed in the metal container. The PE packet is properly sealed by heat, preferably by an electric heat sealer. An alternative procedure is sealing over a candle flame, although this is not so reliable. The bag may also be fastened by tying it as tightly as possible. For extra protection, another plastic bag may be used to contain the bag with the seeds. Many other containers can be used, such as paper bags, cardboard boxes, cotton bags, bottle gourd containers, wooden boxes, porous jars without glaze, plastic or glass bottles without rubber-gasket stoppers. These containers however are not moisture proof. They can be used in places with low relative humidity or when storage period is very short. If the seeds are stored by hanging them above an open wood stove, as is done in some places, these containers could store seeds for a longer period. It blue silica gel (silica gel with cobalt chloride as color indicator) is available, it can be used as an indicator of leakage when a transparent bottle or plastic bag container is used. Dry silica gel is blue and the wet gel is pink. Asmall perforated PE bag of this type of silica gel can be packed with the seeds. During storage ifthe silica gel stays blue, then the seal isperfect; and ifitstarts to turn pink, then there isleakage and the container has to be tightly sealed.

Seed storage

The loss of viability impairs the biological value or function of seeds, which is to protect and nourish the living cells of the embryo, until seedling is established. The cultivator is concerned with the phenomena of seed longevity because he wants high germination and vigorous seedlings from the seed which he has planted. Nursery men concerned with seed viability to get maximum return of it. While scientist accept high longevity of this is breeding stocks and not to loose the value able material through death of seed. For all mankind it is

concerned with seed longevity since the genetic material concerned in seed bank may be essential to our survival in changing ecology of this world in future.

In the tropical climates where both temperature and humidity are high, seed storage often present problem which are lacking in temperate conditions. Seed longevity is affected by several factors as follows.

1. **Seed factor**: Seed storage begins immediately after maturity regardless of where or seeds are held. Viability of seed varies with the crop and it is short lived in onion, beans, cowpea, and capsicum seeds. In heritance of seed longevity is not limited to the species but also to cultivars. Generally healthy, pulpy and well matured seed stored better than immature seed. The moisture content of the seed during storage is no doubt the most influential factor affecting their longevity. It is important to harvest mature relatively dry seeds or to reduce the moisture content of freshly harvested seed soon after harvest.
2. **Storage environment**: The storage requirements for the maintenance of viability vary for different type of seed. Storage temperature and seed moisture content are the two storage condition both relative humidity and temperature are kept low of the two seed moisture content in relatively more than temperature. Temperature are also play in important role in of seed although it does not appear to be a controlling with normal range of biological activity of seed, insect and mold increase as temperature increase. Higher the moisture content of the seed the more they are adversely affected by the temperature. Decreasing temperature and seed moisture content there for is an effective means of maintaining seed quality in storage. Harrington (1972) stated that life of seed halves (1) for every 50°C increase in storage temperature (2) for increase in every one percent of seed moisture. This holds well between 5-14 percent of seed moisture and 0 to 50°C temperature most of the vegetable seed ideal stored at 6-7% moisture content.
3. **Storage container:** A storing of seeds in the containers made up of suitable packing material will prevent the direct contact of seed with the storage environment and this is another approach for retaining viability. The packaging materials used are paper, cotton, metal, plastic, glass and laminated foil. These are selected according to kind and amount of seeds to be packed, during of storage etc.
4. **Storage fungi:** The storage fungi comprise mainly aspergillum and pencilliumspp which affects the seed storability. These fungi will grow successfully at moisture content equivalent to RH of 85% or above. Storage fungi affect the seed by decrease in germination, discoloration, production of mycotoxins, heating and total decay. Chemical treatment with fungicides prevents the attack of storage fungi.
5. **Physiologicaland biochemical changes in seeds during storage:** Among many physiologicalmanifestation of seed deterioration are changed in seed colour, delayed germination, decreased tolerance to sub optimal environmental conditions during germination and storage conditions, reduced germination and seeding growth and increased number of abhoma/seedlings. Biochemical changes includes: 1) increase or decrease in enzyme activity, 2) decrease in oxygen up take, 3) increase in leaching of organic and inorganic constituents from seed, 4) increase in free fatty acid, 5) decrease in total soluble sugar, 6) increase in reducing sugar and decrease in total soluble sugar, 7) decrease in protein and increase in amino acid, 8) changes in carbohydrate, organic acid and protein metabolism.

To conclude, a quality seed is required for better establishment of seeding in field as well as for higher crop production. This could be achieved through

a. Production of seed under ideal climatic and enrich soil condition

b. Supplementation of seed through seed treatment

Proper handling and storage of seed for higher longevity.

The proper handling and storage of seeds for higher longevity depends upon various factors namely storage temperature and relative humidity. The recommended temperature and relative humidity for storages of some vegetable seeds are listed in Table-1.

Table-1: Recommended temperature, relative humidity and approximate storage life of various vegetable seeds

Vegetables	Temperature (°C)	Relative humidity (%)	Approximate Storage life
Beans: Lima in pods	40-45	95	7-8 days
Dolichos lab in pods	32-35	90	2-3 weeks
Beet root	32	90-95	2-3 months
Bitter gourd	33-35	85-90	1 month
Brinjal	50-55	90-95	2-4 weeks
Cabbage, early	32	95-98	3-6 weeks
Cabbage, later	31-32	95-98	4-5 months
Carrot topped	32	95	5-6 months
Cauliflower	32-35	85-95	5-8 weeks
Celery	31-32	92-95	8 weeks
Coriander leaves	32-35	90	5 weeks
Cucumber	50-55	95	10-14 days
Garlic (bulbs) dry	32	60-65	7-8 month
Ginger	55	65	5-6 months
Gourd, bottle	45	85-90	4-6 weeks
Gourd, snake	65-70	85-90	2 weeks
Knol, knol	33-34	90	3 months
Muskmelon, cantaloupe	35-38	85-90	10 days
Muskmelon honeydew	45	85	4-5 weeks
Okra	45-50	90-95	1-2 weeks
Onion, white	34	70-75	4-5 months
Onion red	32	70-75	5-6 months
Pea, green	32	88-92	2-3 weeks
Pepper, sweet(green)	45	85-90	3-5 weeks
Potato	38-40	85	7-8 months
Pumpkin	35-60	70-75	3-5 months
Radish, topped	32	88-92	3-5 weeks
Spinach	32	95	10-14 days
Squash, summer	50	95	1 week
Squash, winter	50-60	60	3-4 months
Sweet potato	55	90	4-5 months
Tapioca root	32-35	85	5-6 months
Tomato, ripe	35-45	85-90	5-7 days
Turnip	32	90-95	2-4 months
Watermelon	45-60	80-90	2 weeks
Yam	80	60-70	3-5 weeks

Conclusion

High-quality seed is one of the most important factors affecting the success of a crop. Lack of high-quality seed, which is brought on by bad seed production technology, a lack of appropriate quality control, and improper handling of post-harvest seeds, is a serious problem in developing countries. The quality of the seeds must be maintained for higher-quality harvests. More efficient, practical, and cost-effective postharvest handling and seed storage methods must be created. The primary objective of research should be to translate knowledge into beneficial outcomes for agriculture. This chapter has addressed the importance of healthy seeds, factors that affect seed quality, post-harvest seed storage methods, techniques and safety precautions for their quality assessment for the maintenance of good quality seeds to meet international standards with a focus on developing countries' needs. A more advanced and sensitive technology might be used to do this, along with careful observation of how seeds interact with their surroundings.

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Post Harvest Handling of Seeds

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

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

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

Concept of Separation Processes:

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

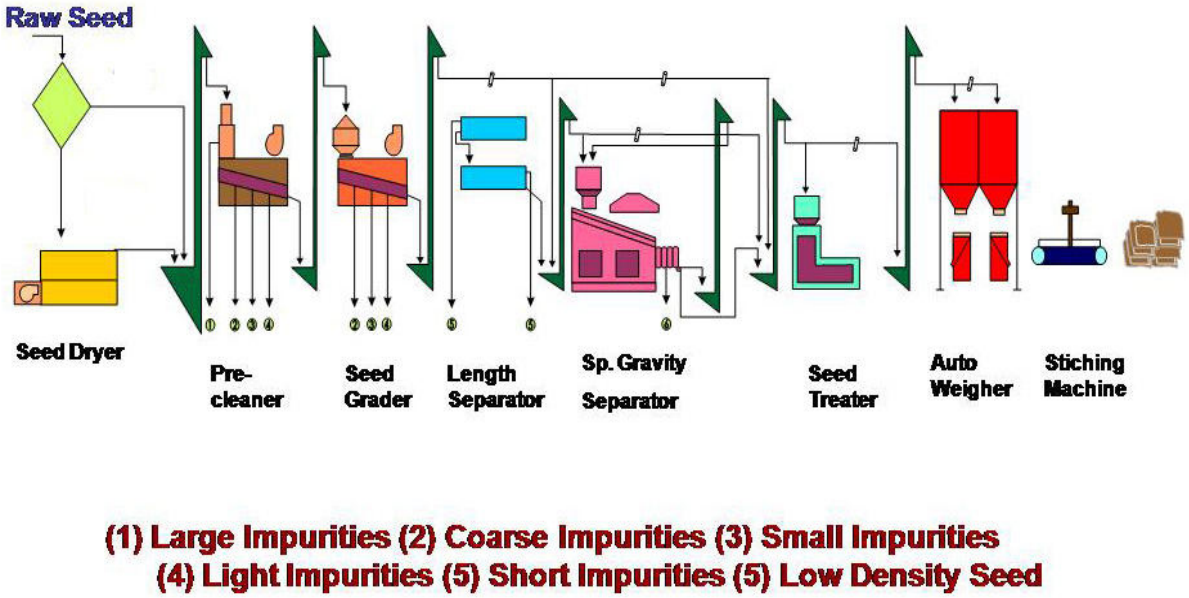

Fig-1: Flow diagram of modern seed processing

Methods of Seed Separation:

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

Air-Screen Cleaner:

The air-screen cleaner is the most widely used machine. It is an essential unit operation in seed processing plant. The simplest mechanical method of separating particulate solids, the class to which most agricultural seeds and food grains belong, is by passing than over screens which are stationary or reciprocating and are set at a slight downward slope, so that small particles will pass through and larger materials will tail over them. In combination with air-fans or blowers, the screen machine provides adequate conditioning for some seed crops. Such machines work by taking advantage of dimensional and aerodynamic differences. Agricultural screens are constructed of perforated metal or woven wire mesh. Hole shapes in perforated screens are usually round, triangular, oblong or rectangular, Openings in wire screens are square or rectangular, their size being represented by mesh numbers. Round hole screens are identified by a number denoting diameter of the perforation. In India, these numbers indicate the diameter in millimeters. Rectangular or oblong holes in perforated screens are identified by two numbers describing the width and length of the slot. Selection of the screen depends on the seeds being handled. Screen opening sizes used for different crops have been prepared and are available in literature. Screens with various sizes and shapes of holes drop some particles and retain others depending mainly on the width and thickness of particles and, to a lesser extent on their length. Pneumatic separators or air columns exploiting aerodynamic differences are used to remove dust, chaff or other light contaminants. The air system in air-screen machine operates in this manner. As a finishing machine it can remove light, immature, shriveled or damaged

seeds from already cleaned good seed lots. Air screen combinations are extensively used in grain combining and threshing.

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

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

Principles of operation:

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

Length Separator:

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

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

Specific Gravity Separator:

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

Seed Refining:

To further refine the seed, machines have been developed to take advantage from additional differences in physical properties. The electrostatic separator exploits the difference in the electrical characteristics of the seeds and contaminants. The quality of separation depends on the relative availability of the components in the seed mixture to conduct electricity or to hold electrical charge on surface. A spiral separator senses the ability of components to roll. This is very simple machine and operates completely by gravity. It has no moving parts and needs no prime mover. The endless draper belt separator utilizes surface texture differences to separate rough seeds from the smooth ones. A magnetic separator requires certain pre treatment of the feed mixture. Iron power or a magnetic fluid is added. Variation in seed coat characteristics is utilized. The iron is selectively adsorbed by rough, broken, cracked porous or sticky components making them more reactive than the smooth components. A colour separator acts on differences in reflective properties. The components of the mixture must be cingulated for individual sensing by the photoelectric cells. To scale up the throughput multi-channel machines are required.

New Emerging Technologies:

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

appears a need to develop expert systems for modern seed processing and once a system is made available, the performance and the status of an average worker can be raised to the level of an expert.

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Management of Seed Storage

M. K. Vishwakarma[1] and Ekta kumari[2]
Seed Processing Engineer[1] and Sr. Seed Analyst[2], NSRTC, Varanasi

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

Factors Affecting Seed Longevity in Storage

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

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

Relative humidity and temperature during storage: Relative humidity and the temperature in the air of the seed storage room are the major environmental factors influencing the storage life of the seeds. Low relative humidity makes the air thirsty of water and it picks up the unwanted moisture from the seed. Hence the seeds are kept dry in low humidity condition. Seeds achieve a rather specific and characteristics moisture content, termed as equilibrium moisture content,

when subjected to a given combination of atmospheric relative humidity and the air temperature. This results due to the hygroscopic nature of the seeds. Fortunately, the establishment of moisture equilibrium in seeds is a time dependent process and it does not occur instantaneously. Therefore, the diurnal fluctuations in the relative humidity have little effect on moisture content.

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

Temperature Control:

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

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

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

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

Classification of moisture and heat removal systems configuration

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

Refrigeration: Refrigeration is the household term. It is a process by which the heat is made to flow from lower to higher temperature, i.e., against the natural heat transfer process. It is the only method to achieve and maintain low temperature on long term basis. The medium employed to absorb heat is the refrigeration agent or simply refrigerant. Mechanical refrigeration systems are based on the ability of liquid heat as they vaporize. The vaporizing temperature of the liquid can be regulated by controlling the pressure at which the liquid vaporizes. In closed systems, the vapour is condensed back into liquid and thus used over and over again to provide a continuous flow of liquid for vaporization. Of all the fluids currently used as refrigerant, the one nearest to idle general purpose refrigerant is refrigerant-12 or R-12. It has a saturation temperature of –29.8°C. It can be stored as a liquid at ordinary temperature only under pressure in heavy steel cylinders. A typical mechanical refrigeration system contains the following parts: (1) An evaporator to provide heat transfer surface through which heat moves from the space being refrigerated into the vapourising refrigerant; (2) a suction line to convey the refrigerant vapour from the evaporator to the compressor; (3) a compressor to heat and compressor the vapour; (4) a hot gas or discharge line to carry the high-temperature, high-pressure vapour from the compressor to a condensor; (5) a condenser to provide heat transfer surface through which heat passes from the hot gas to the condensing medium; (6) a receiving tank to hold the liquid refrigerant for future use; (7) a liquid line to carry the liquid refrigerant from the receiving tank to the refrigerant metering device; and (8) a refrigerant

metering device to control the flow of liquid to the evaporator. The typical vapor-compression system is divided into a low and a high-pressure side. The refrigerant metering device, evaporator, and suction line constitute the low pressure side of the system; the compressor, discharge line, condenser, receiving tank, and liquid line constitute the high pressure side of the system. A mechanical refrigeration system that will cool at a rate equivalent to melting one tonne of ice in 24 hours is said to have a capacity of one tonne refrigeration. The capacity of the compressor must be such that the vapor is drawn from the evaporator at the same rate at which it is produced.

Controlling Humidity:

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

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Recent advances in hybrids seed production of vegetable crops

B. S. Tomar
Division of Vegetable Science
ICAR-Indian Agricultural Research Institute, New Delhi-110012

The Global Hunger Index (GHI), 2016, report ranked India 22nd amongst leading countries and having GHI score of 28.5 which indicates a serious hunger situation. India is one of the fastest growing countries in terms of population with an estimated population of 1.31 billion. It is estimated that at the rate of 1.2 percent annual population growth by 2050, Indian population will be the highest (1.7 billion) in the world. India is second largest populous country with an estimated population of 1.31 billion after China. It is estimated that at the rate of 1.2 percent annual population growth by 2050, Indian population will be the highest (1.7 billion) in the world. According to International Food Policy Research Institute (IFPRI), Washington 2016, an estimated 15.2% of the citizens in India are malnourished and not getting enough quantity of food (quantity and quality). In India per capita land resources (0.121 hectare) are shrinking due to the tremendous pressure of the population growth, therefore, it is very imperative to ensure the higher production and productivity per unit of area. Vegetable crops are more productive than other crops, which have potential of providing more food per unit time and land area. According to the study of the Indian Medical Research Council (ICMR), New Delhi and the National Institute of Nutrition, Hyderabad (NIN), changing food habit and limited availability of vegetables is considered responsible for malnutrition in our country. To ensure good health it is recommended that at least 300g of vegetable should consumed as a part of balanced diet, comprising 125g leafy vegetables, 75g other vegetables and 100g root per person every day. Vegetables provide all the nutrients ingredients viz., vitamins, minerals and protein that are essential for balanced diet. The presence of good amount of vitamins and minerals in vegetables makes them protective food. Many vegetables carries good amount of nutraceutical properties and having capabilities to ensure good health.

Although, India is the second largest producer of vegetables with annual production of 162.9 (NHB, 2015) million tonnes but we are still far behind in production and average productivity as compared with China. The per capita gross availability of vegetable in India is 365g/day whereas per capita net availability of vegetable (25% loss + 5% exports and processing) is 256g/day (Horticultural Statistics at a Glance, DAC&FW, 2015). Thus, in order to feed rapid growing population with the limited resources there is a need to enhance the vegetable productivity (17.3 MT/ha) which is less than the average world vegetable productivity (19.6 MT/ha) (NHB, 2015).

Therefore, to increase production and average productivity per unit of area in vegetable crops, hybrid varieties can play a vital role due to their high yield potential, early maturing, superior quality, disease and pest resistance attributes. The steady increase in productivity can be achieved through the use of quality seeds with built in inbred and hybrid vigour, coupled with the application of modern vegetable cultivation technologies and sound government policies. Thus, hybrid vegetable technology is one of the better options particularly due to the

fact that full potential of hybrids in vegetable crops has not been exploited. The main reason for low productivity in vegetables and less commercialization of hybrids in India is certainly due to the non availability of quality seed of improved hybrids and the second reason could be very high cost of hybrid seed of different vegetables like tomato, sweet pepper, chilli and major cucurbits.

Genetic basis of heterosis:

Heterosis, or hybrid vigor, refers to the superior performance of the hybrids relative to the parents. Utilization of heterosis has tremendous potential for increasing productivity of vegetable crops globally.

There are two possible causes of heterosis viz.

a) **Dominance hypothesis:** It is based on the assumption that hybrid vigour results from bringing together female dominant genes. According to this theory, genes that are favourable for vigour and growth are dominant, and genes that are harmful to the individual are recessive. The dominant genes contributed by one parent may complement the dominant genes contributed by the other parent, so that F_1 will have the more favourable combination of dominant genes, than either parent.

b) **Over dominance hypothesis:** According to this hypothesis hybrid vigour on the basis of heteozygosity is superior to homozygosity. The overdominance hypothesis assumes that the heterozygous condition of the alleles at a single locus is superior to either of the homozygous conditions of the alleles at that locus. Thus heterozygosity is the cause of heterosis, while homozygosity resulting due to inbreeding causes inbreeding depression.

Exploitation of hybrids vigour in vegetable crops in India:

In India the first F_1 hybrid variety 'Pusa Meghdoot' of bottle gourd was developed by IARI in 1971. It was in 1973 when Bangalore based Indo-American Hybrid Seed Company from the private sector launched its first successful hybrid in tomato (Karnataka) and capsicum(Bharat) in India, amply demonstrating to the Indian farmers, good returns possible for the money invested in hybrid seeds. The emphasis has been laid out in the development of hybrids and testing under the All India Coordinated Improvement Project on vegetables during mid 1980's and has resulted the identification and release of large number of F_1 hybrids in various vegetable crops. There are number of F_1 hybrids developed by public sector organization which are popular and multiplied by NSC at national and SSC at state level (table 1) and many private sector companies through MTA.

Table: 1 Public sector hybrids in vegetable crops

S.No.	Crop	Hybrids	Source
1.	Tomato	Pusa Hybrid-1, Pusa Hybrid-2, Pusa Hybrid-4, Pusa Hybrid-8, Pusa Divya (Kt-4)	IARI, Delhi
		Arka Rakshak, Arka Ananya, Arka Samrat, Arka Shreshta, Arka Vishal, Arka Vardan, Arka Abhijit	IIHR, Bengaluru
		Kashi Abhiman	IIVR, Varanasi
		Pant Hybrid-1, Pant Hybrid-2, Pant Hybrid-10, Pant Hybrid-11	GBPUAT, Pantnagar

		Rajashree, Phule Hybrid-1	MPKV, Rahuri
2.	Brinjal	DBHL-20, Pusa Hybrid-5 (Long), Pusa Hybrid-6 (Round), Pusa Hybrid-9, Pusa Anupama(Kt-4)	IARI, Delhi
		Arka Navneet	IIHR, Bengaluru
3.	Chilli	CH-1, CH-3	PAU, Ludhiana
		Arka Meghana, Arka Harit, Arka Sweta	IIHR, Bengaluru
		Kashi Early, Kashi Surkh	IIVR, Varanasi
4.	Sweet pepper	Pusa Deepti, KTCPH-3	IARI
5.	Cucumber	Pusa Sanyog	IARI
6.	Bitter gourd	Pusa Hybrid-1, Pusa Hybrid-2	IARI, Delhi
	Bottle Gourd	Pusa Hybrid-3	IARI, Delhi
		Kashi Bahar	IIVR, Varanasi
		Pant Sankar Lauki 1	GBPUAT, Pantnagar
		Narendra Sankar-1	NDUAT, Faizabad
7.	Muskmelon	Pusa Rasraj,	IARI, Delhi
		Punjab Hybrid-1,	PAU, Ludhiana
8.	Pumpkin	Pusa Hybrid-1	IARI, Delhi
9.	Summer squash	Pusa Alankar	IARI, Delhi
10.	Watermelon	Arka Jyoti	IIHR, Bengaluru
11.	Cauliflower	Pusa Kartik Sankar, Pusa Hybrid-2, Pusa Snowball Hybrid-1	IARI, Delhi/ R.S Katrain
12.	Cabbage	Pusa Cabbage Hybrid-1	IARI, Delhi
13.	Carrot	Pusa Vasudha, Pusa Nayanjyoti	IARI, Delhi/R.S Katrain
14.	Onion	Arka Lalima, Arka Kirtiman, Arka Bhima	IIHR, Bengaluru
15.	Okra	Kashi Bhairav	IIVR, Varanasi
16.	Ashgourd	Pusa Shreyali and Pusa Urmi	IARI, Delhi

Breeding behaviour in vegetable crops: Successful seed production depends upon breeding system (self-pollinating, cross-pollinating and often cross-pollinating), life cycle (annual, biennial and perennial), sex form (hermaphrodite, monoecious, dioeciuous) and compatibility (self-fertile, self-incompatible) of vegetable crops (table 2).

Table: 2 Breeding system, sex form life cycle and compatibility of Vegetable crops

Breeding system	**Sex form**	**Life cycle**	**Compatibility**	**Vegetable crops**
Self-pollinating	Hermaphrodite	Annual	Self fertile	Brinjal, Chilli, Sweet pepper, Tomato, Cowpea, Lettuce, hyacinth bean
Often cross-pollinating	Hermaphrodite	Annual	Self fertile	Okra, Lima bean

Highly cross-pollinating	Hermaphrodite	Annual	Self fertile	Palak, Amaranth, Chenopodium, coriander
	Hermaphrodite	Biennial	Self-incompatible	Cauliflower, cabbage, Knol-Khol, Broccoli, Brussels Sprouts, Kale, radish, Beet root, Turnip
	Hermaphrodite	Biennial	Self fertile	Carrot, onion, celery
	Hermaphrodite	Perennial	Self fertile	Artichoke
	Monoceious	Annual	Self fertile	Cucumber, Bitter gourd, Bottle gourd, Luffa, Pumpkin, Squash, Watermelon, Round melon
	Andromonoecious	Annual	Self fertile	Muskmelon
	Gynoecious	Annual	Self fertile	Cucumber, Bitter gourd
	Dioecious	Perennial	Self fertile	Pointed gourd, Ivy gourd, Asparagus
	Dioecious	Annual	Self fertile	Spinach

Source: Vishnu swarup (2006).Vegetable Science and technology in India

Principles of vegetable seed production:

1. **Isolation Distance:** The hybrid seed production field must be isolated from other varieties of the crop, wild relatives and other cultivated species of crop to ensure the production of genetically pure seed. Several vegetable crops being highly cross pollinated; therefore isolation distance both for foundation and certified seed production should be maintained as per the seed production standards. Proper isolation is thus required to maintain genetic purity of a variety. Isolation between cross compatible varieties is achieved following ways.
    a) **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.
    b) **Isolation by distance:** 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).
2. **Choice of season and areas of seed production:** Seed crop should be raised in such a seasons which remain dry at the time of seed maturity and seed extraction. Locations are also important in seed production with reference to seed yield and quality of seed. To harness the advantage of climate, private sector seed companies are organizing their seed production in these areas. The Punjab & Haryana, U.P Jalana (Aurangabad) in Maharashtra, Ranibenur and around Bangalore in Karnataka, Nandyal Valley in A.P., are the main areas of seed production for muskmelon and cucumber in India.

3. **Roguing:** Roguing 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. The off-types may occur in a crop due to diversity of the morphological types within a crop may be wide. The examples are predominantly cross-pollinated vegetable crops like cole crops, cucurbits and onion) shows high morphological diversity than self-pollinated (e.g. peas, tomato, fenugreek) crops. This is the basic reason that varieties of self-pollinated crops are generally more uniform and stable than varieties of cross pollinated crops. It is always easier to conduct intensive rouging in breeder seed plots than in commercial seed production.

   Stages of rouging:

   a) **Before flowering:** Off types are detected on the basis of vegetative characters like plant growth, foliage morphology, colour etc.
   b) **Flowering stage:** Early and late varieties can be easily identified on the basis of sex expression and sex ratio in cucurbits and flower initiation time in solanaceous crops.
   c) **Fruit developing stage:** Trueness to type of developing fruit is checked and on the basis off -type plants are rouged out. Fruit shape, size, colour, colour of ripen fruit (green, yellow, red) should be taken into consideration for tomato.
   d) **Maturity stage:** At this stage it is essential to remove plants showing late maturity of fruits in the early variety and vice versa. Disease effected plants, which is seed borne should be carefully removed and destroyed.

4. **Threshing and seed extraction**: Seed should be extracted from dry fruits or from fruits in which the seeds are wet at the time of extraction. Threshing can be done by hand or machines. 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.

   Methods of Seed extraction:

   a) **Dry Method**: The fully matured and dried fruits are harvested and dried in the sun for 2-3 days. After extraction of seed, the seed are dried in the sun between 8.00-11.00 Am and 2.00-5.00 Pm to bring out the original moisture content. Eg. Chilli, Okra, Sponge gourd, Ridge gourd, etc.
   b) **Wet Method**: This method is employed for seeds extraction of tomato, brinjal, cucumber, muskmelon, watermelon, ash gourd, bitter gourd, round melon and long melon. In wet method, the seed extraction done by three ways :
      i. Acid Method: In this method the fully ripened matured fruits are harvested and crushed to pulp. The pulp is taken in plastic container or wooden container or cement tub of convenient size and the commercial HCL added. The acid and pulp are mixed thoroughly and kept for as such time. During this period, corrosiveness of the acid removes the mucilage adhering to the seed and makes the seed free of pulp. Then, the seeds are washed 4-5 times thoroughly with water to make free of acid, otherwise the remanants of the

acid spoils the embryo of the seed. The seed extraction is quicker in this method. Seed are also bright in colour with good germination ability and free from fungal attack. The different extraction methods found that the seed recovery percentage was higher in acid method irrespective of varieties. Germination was highest in 2.5% HCL with 30 minutes soaking duration.

ii. Fermentation Method: The fruits are crushed in a non-metalic container and kept as such for fermentation for 2-3 days. It has been observed that 2 days fermentation of fruits is the best for getting quality seed. During fermentation the seeds get detached from the adhering pulp and settles to the bottom of the container. The seeds are separated, washed thoroughly and dried under shade to the desired moisture level. The seed recovery is less compared to other method of extraction. The seeds become dull coloured due to fermentation of the pulp and also due to the fungal load in the seeds. *In situ* germination may occur due to long period of fermentation. These are method used in vegetables like, Tomato, Brinjal, Cucumber, Water melon, Musk melon, etc.

iii. Alkali Method: Fully ripened matured fruits are harvested and crushed to make pulp. In Tomato, to hasten the fermentation process 0.5% sodium bicarbonate (500 g dissolved in 10lit of warm water is added to the pulp and allowed to remain for a day. Then, the seeds are separated and washed free of alkali with water.

5. **Seed Standards**: It refer to the field inspection of the harvested produce as well as the manner of harvesting, transporting, processing and packing. Unless, a seed certification agency keeps track of harvested produce until it is packed and sealed the identity of the lots cannot be assured. It is, therefore, necessary that the seed certification agency should lay down standard for processing plants. In addition, field and seed standards, such as isolation distances, inseparable other crop seeds, weeds, plants affected by seed borne diseases, genetic purity, percentage of pure seed, other crop and weed seeds, inert matter, moisture content, germination and insect damage, should be prescribed for successful accomplishment of the certification.

Techniques of hybrid seed production in vegetable crops:

Seed production through manual pollination method on commercial scale is only economically feasible in the hybrids of vegetable crops like tomato, eggplant, and cucurbits (bottle gourd, watermelon, pumpkin etc.) where large numbers of F_1 seeds can be obtained from one manually pollinated crossed fruits. Nevertheless, various advanced hybrid seed production techniques like, functional male sterility in tomato and brinjal, stable genic and sporogenous male sterility with marker character in watermelon and muskmelon etc. can be utilized in these vegetables to reduce cost of F_1 seed production. The functional male sterility has been exploited for hybrid seed production of tomato cv Pusa Divya under poly house condition by Manjunath (2009). Hybrid seed in tomato, brinjal, capsicum and chilli are produced through hand emasculation and pollination; bottle gourd, bitter gourd and pumpkin through protection of female flower and hand

pollination (Flemine, 2010; Jat, 2011; Behera *et. al*, 2015); cucumber through natural pollination in case of gynoceious seed parent (Munshi *et al*., 2015); onion, cabbage and cauliflower hybrid seed are being produced by utilizing the CMS and SI system respectively where as the hybrid seed of summer squash is produced through the use of ethephon for inducing the pistilate flower and natural pollination. Several techniques and method have been developed for the hybrid seed production of vegetable crops, however only selected techniques are being utilized for hybrid seed production on the basis of their commercial and economically feasibility (table 2).

Cryopreservation of pollen in liquid nitrogen at -196°C offers rich promise to the hybrid seed production of vegetables where low pollen production and low pollen viability is one of the major. This method can provide a constant supply of viable and fertile pollen and can also allow supplementary pollinations for improving seed set. Thus, use of cryo-preservation for storing pollen can be effectively used for hybrid seed production which helps in reducing cost of production, genetic integrity and germplasm security.

Table 4: Method/systems of hybrid seed production of Vegetable crops

S.No.	Hybrid seed production mechanism	Commercially exploited crops
1.	Hand emasculation and manual pollination	Tomato, Brinjal, Sweet pepper, Okra, Chilli
2.	Pinching of staminate flowers and hand pollination	Bitter gourd, Bottle gourd, pumpkin
3.	Removal of staminate flower + emasculation + hand pollination	Watermelon and muskmelon
4.	Functional male sterility and hand pollination	Tomato, Brinjal
5.	GMS + bee pollination	Chilli
6.	CMS + natural pollination	Capsicum, onion, cabbage, carrot, radish
7.	Self Incompatibility and natural pollination	Cauliflower , broccoli
8.	Gynocecism and natural pollination	Cucumber , bitter gourd
9.	PGR and natural pollination	Squash's
10.	Detasseling + wind pollination	Sweet corn, baby corn

Mechanisms for hybrid seed production in vegetable crops:

1. **Hybrid seed production using gynoecious sex form:** Gynoecy condition where all the flowering nodes in the primary, secondary and tertiary branches bear pistillate flowers in the leaf axils is by far the most important sex form which has made phenomenal exploitation of hybrid vigour possible in cucumber, bittergourd and muskmelon (Munshi *et al*., 2017). The commercial production of gynoecious cucumber seed was made possible only when it was discovered that gynoecious inbreds could self reproduce if a growth regulator is applied to induce male flower formation (Robinson, 1999). Peterson and Anhder (1960) for the first time discovered the effect of gibberellic acid (1500-2000 ppm) on promotion of male flower formation in cucumber. A problem observed with gibberellic acid application is that

different gynoecious lines vary in response to GA application and, in some cases, the number of induced male flowers was not sufficient for hybrid seed production. Additionally, GA applications typically cause excessive stem elongation or malformed male flowers (Robinson, 2000). Because of the erratic male flower induction by use of gibberellic acid, application n of silver compound such as silver nitrate (250-400 ppm) is done to induce male flowers. Silver ions inhibit ethylene action and thus promote male flower formation in gynoecious cucumber plants (Beyer, 1976). However, due to phytotoxic effects of silver nitrate such as burning of plants, silver thiosulphate (400 ppm) is now widely used by seed producers for the maintenance of gynoecious cucumber lines. It induces male flowering of cucumber plants over a longer period and is less phytotoxic compared to silver nitrate.

Hybrids of cucumber are produced mainly by crossing gynoecious lines with monoecious lines. Though, other systems of producing gynoecious hybrid seed such as gynoecious × gynoecious have been proposed but gynoecious × monoecious hybrids are still the most widely grown. The hybrids produced by the cross of a gynoecious and monoecious line resulted in hybrid vigor and a high degree of female sex expression, with uniform and concentrated fruit formation, which was especially advantageous for mechanical harvest (Robinson 1999, 2000).

For hybrid seed production female and male rows are planted in 4:1 ratio. The female (seed parent) bear only female flowers and pollination in done by insect (honeybee). To ensure the good fruit and seed recovery, the sufficient population of honeybee 1 to 1½ colony of medium size has to be kept at the boundry of seed production plot to boost the amount of crossing. The parental lines i.e. male parent maintained by selfing (mixed pollination) and rouge out undesirable plants before contamination take place. The female lines i.e. gynoecious lines maintained by inducing the staminate flower through the sprays of silver nitrate 200 ppm at two to four true leaf stage and then selfing is carried out. It was observed that 10-11 male flowers appear per 100 nodes.

2. **Hybrid seed production using male sterility system:** Genetic male sterlity systems have been utilized for commercial hybrid production in muskmelon (Punjab Hyb.-1). For hybrid seed production, the male sterile line used as female parent. Since genetic male sterile line is maintained in heterozygous forms, 50% fertile plants are to be removed at flowering. The other 50% having non-dehiscent empty anther are retained in female rows. The female and male are grown in 4:1 ratio. However, to maintain the good plant population in female rows it is suggested that seed parent should be sown with double seed rate. It is also advised that female line seedling should be raised in polythene bags and transplanted at flower appearance in order to avoid the fertile plants in female rows. The pollination is done by honey bees and 1 to 2 medium sizes hives are good enough to ensure the good pollination and fruit set at female row. The male sterile line is maintained in heterozygous form by crossing with maintainer line under adequate isolation distance or under cover.

Among the genetic emasculation tools, both genetic male sterility (GMS) and cytoplasmic genetic male sterility (CGMS) have been employed in hybrid seed production of chilies. During hybrid seed production, the heterogeneous population consisting of male fertile and male sterile plants and the male parent of interest are planted in alternate rows in

the plot in isolation (400-500 m from other commercially grown chili crop). After flowering one can easily distinguish the sterility/fertility by observing for pollens through nail test (shed pollen from the flower on to the nail and if whitish powder like particles are seen- it indicates male fertility, and if no powdery substance is seen, the plant is male sterile) or through pollen viability test under the microscope. The male fertile plants are removed from the plot and the remaining plants from the heterogeneous population are female fertile (male sterile). The seeds developed on the male sterile plants after open pollination from the male fertile plants (male parent of interest) are the F_1 hybrid seeds. After completion of hybridization programme, all the non-crossed (untagged) fruits developing/developed through natural cross-pollination (NCP) on female plants are removed, which facilitates vigorous development of crossed fruits and seeds. In case of CMS/GMS based hybrid seed production, this practice is not required, as all the fruits developed on the male sterile plants will be crossed fruits, provided recommended isolation distance is maintained. The S-cytoplasm of this line is usual source of CMS used in the production of hybrid seeds of chili pepper. Using these male sterile lines, hybrid cultivars (Kashi Surkh from IIVR, Varanasi; Arka Meghana, Arka Sweta and Arka Harita from IIHR, Bangalore) were identified for commercial exploitation.

3. **Hybrid seed production through chemical sex expression:** The hybrid seed can also be produce in cucurbits by the application of chemicals for attaining the sex of cucurbits. Specific chemicals are known to induce femaleness and maleness as desired. The spraying of ethrel (2-choloro-ethyl-phosphonic acid) 200-300 ppm at two and four true leaf stage and another at flowering is useful for inducing the pistilate flower successively in first few nodes on the female in bottle gourd, pumpkin and squash for F1 seed production, Verma et al., 1985.The row of male parent is grown side by the side of female and natural cross pollination is allowed. In the absence of insect, hand pollination is possible when two sexes are separate. Four to five fruit set at initial nodes are sufficient for hybrid seed. The complete suppression of male flowers in squash can be achieve at higher concentration of (400-500 ppm) of ethrel applied twice and has made hybrid seed production comparatively easier and nearly 56% of total squash seed produced in USA is of F1 hybrid. The other chemicals like GA3, (10-25 ppm) in cucumber, MH-(100 ppm), ethephone (600 ppm) in squash induces female flowers.

Hybrid seed production under protected conditions:

Quality hybrid seed production of vegetable crops face tremendous challenges like lack of sufficient isolation, insects, diseases and a virus free environment in the production of disease free, healthy and genetically pure seed for commercial cultivation. Compared to conventional practices, protected cultivation can deliver higher seed yield with better quality (Tomar and Jat, 2015). Insects and viruses are the most devastating problems for quality seed production in most of the vegetable 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.

Insect proof net house is suitable for hybrid seed production of tomato, sweet pepper, chilli, okra, brinjal and cucurbits as compared to open field condition (Jat *et al*., 2015; 2016). Semi-climate controlled greenhouse is suitable for hybrid seed production of indeterminate type

varieties and hybrids of standard tomato, cherry tomato, sweet pepper and parthenocarpic cucumber varieties. Seed yield of such crops can be 3-4 times more compared to their open filed cultivation (Jat *et al*., 2017). Similarly, naturally ventilated green house is also suitable for hybrid seed production, where the seed yield is usually 2-3 times more over open field, but the cost of seed production is only 1/3 of the seed produced under semi-climate controlled green house condition (Singh and Tomar, 2015).

The major advantages of hybrid vegetables seed production under protected conditions are:

1. Higher seed yield (generally 2-4 times more) and seed quality as compared to open field
2. Requirement of large isolation distance in cross pollinated vegetables can be minimized under protected conditions.
3. Problem of synchronization of flowering in parental lines can be minimized.
4. Maximum plant population can be maintained along with appropriate ratio of male and female parents for higher hybrid seed yield.
5. Seed production under adverse climatic conditions is possible where it is not possible in open field conditions.
6. 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.
7. Training, pruning and hand pollination practices are very easily manageable under protected conditions compared with to field seed crop.
8. Hybrid seed production can also be possible even under saline and acidic soil conditions by using soil less media.
9. Virus free healthy seedling production of the parental lines is possible under protected conditions.
10. Emasculation of female parents is not required as there are no insect pollinators inside the protected structures.
11. Seed crops will not be damaged by un-seasonal rains at the time of their maturity unlike open field seed crops.
12. Off type plants, objectionable weeds or plants affected by designated diseases cannot pose problems in the seed crops raised under protected conditions.
13. Seed viability and seed vigour could be extended through better nutrient management in seed crops under protected conditions

Genetic purity testing of hybrid seed:

Usually, grow-out-trails (GOTs) have been widely used to evaluate the genetic purity of hybrid seed in a variety of crops (Moorthy et al., 2011; Liu et al., 2004). GOT involves the comparison of morphological traits of plants raised from seeds of test sample, with that of genuine samples throughout the crop's growing season. However, GOT is time consuming, laborious and crop season specific process, which delays seed certification program. Therefore, biochemical markers based on Isozymes differences have been utilized for seed genetic purity testing but these markers are limited in number, affected by environment, plant tissue type and stage. Hence, a precise and competent method is required to make hybrid seeds testing a rapid and cost effective method. One such alternative is the DNA marker, which detect the level of

admixtures in a seed lot based on the established variations between the cultivars at the level of nucleotide sequences. Such differences remain unaffected across different growth stages, seasons, locations and agronomic practices making varietal identification and genetic purity testing more precise, reproducible and objective. Recently, several molecular marker systems such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter-Simple Sequence Repeat) and SSR (Simple Sequence Repeat) have been extensively employed in seed genetic purity testing in field crops. Among these, the SSR markers are popularly used for assessing the hybrid purity in different crop plants because of the simplicity, rapidness, reproducibility and cost effectiveness compared to other types of markers. As SSR markers are co-dominant in nature, heterozygosity of the hybrids can be easily determined by the presence of polymorphic parental alleles, which make them suitable marker for testing the hybrid purity against the admixture of selfed seeds as well as off types. Application of SSR markers for assessing seed purity has been reported in vegetable crops like bunching onion (Tsukazaki et al., 2006), cabbage (Liu et al., 2007a), tomato (Liu et al., 2007b), artichoke (Bianco et al., 2011), chili (Mongkolporn et al., 2004), melon (Liu et al., 2006), squash (Ferriol et al., 2003) and cauliflower (Zhao et al., 2012). With the benefits of time saving, precision, less labour-consumption molecular markers are becoming vital tools for cultivar identification and seed quality control in many crops.

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Strategic approach for improvement in vegetable crops- An Overview

B. S. Tomar
Division of Vegetable Science
ICAR-Indian Agricultural Research Institute, New Delhi-110012

Introduction

Classical plant breeding is instrumental in meeting the growing food demands because of increasing population worldwide. In India, application of traditional breeding tools in combination with modern agro-techniques was the main force behind green revolution and turning India into a food surplus country. Similarly, in vegetable crops, the traditional plant breeding approaches have yielded immense results in improving productivity, quality and stress tolerance in several vegetable crops. Biotechnology is a rapidly developing area of contemporary science. It can bring new ideas, improved tools and novel approaches to the solution of some persistent, seemingly intractable problems in food crop production. Given the pressing need to enhance and stabilize food production in response to mounting population pressures and increasing poverty, there is an urgent need to explore novel technologies that will break traditional barriers. A number of biotechnology applications and approaches are being employed to enhance of agricultural production in many countries. Application of modern DNA based techniques in improvement of crop plants have started in the last three decades. However, in vegetable crops these tools were rarely used till the last 15-20 years. However, genomic resources were developed in several important vegetable crops in the last ten years and thereafter the molecular tools have been used widely in improvement of these nutritionally rich vital crops. Application and availability of in vitro based tools are known for a long time. However, limited application of these tools in crop improvement is mainly because of requirement of substantially long time period in developing end products using these tools. However, availability of huge genetic resources in the wild and related species of different crops offers an unique opportunity to broaden the genetic base of the cultivated species. Application of in vitro based tools play an important role for harnessing desirable traits from wild to cultivated genotypes. Advancement in development of haploids and doubled haploids in the last decade created a huge interest among plant breeders and several multinational seed companies in rapid development of inbreds in highly cross-pollinated crops like vegetable Brassicas, carrots, onion, cucurbits and several other vegetables. Besides, it was evident that when the in vitro based tools are combined with modern molecular based techniques they may hasten the improvement programme in a rapid pace. Besides, it was also possible to create the genetic diversity among the crops we lost because of genetic erosion as a result of intensive selection for few desirable traits. In this chapter we discuss the modern in vitro based and molecular tools available in different vegetable crops and their application in meeting the future and emerging challenges in vegetable crops.

In vitro based techniques

Plant tissue culture-based techniques are widely used in improvement of different crops including vegetables. These techniques are now recognised for their application in generation advancement, transfer of desired genes from secondary or tertiary gene pool and development of novel male sterility systems in vegetable crops. Application of in vitro techniques in mass multiplication of vegetable crops is very limited as most of the vegetables are short duration in nature and sexually propagated through seeds. However, development of virus frees planting material on garlic and shallot. Therefore, the application of embryo rescue, somatic embryogenesis, in vitro pollination, flowering and fertilization, somaclonal variation as well as protoplast and somatic hybridization, development of doubled haploids are discussed in this section.

Role of molecular markers

The use of morphological markers has continued till today for characterization of several crops including horticultural crops with the Mendel's principles of inheritance by following visible traits in the progeny of sexual crosses. Genetic markers play an essential role today in the study of variability and diversity, in the construction of linkage maps, and in the diagnosis of individuals or lines carrying certain linked genes. Within this context, the limitations of morphological markers became quickly apparent. They tend to be restricted to relatively few traits, display a low degree of polymorphism, are often environmentally variable in their manifestation, and can depend on the expression of several unlinked genes. Furthermore, some may affect plant viability or seed set, distorting gene frequencies in the progeny. Morphological markers were largely supplemented by biochemical markers, particularly isoenzymes that could be easily scored by electrophoresis. The limitations of isoenzymes as markers, in particular both the limited number of polymorphic enzymes that can be conveniently stained and the environmental effects on expression pattern, were apparent already twenty years ago. The shortcomings drove the development of markers based on DNA polymorphisms. These marker types generate ''fingerprints,'' distinctive patterns of DNA fragments resolved by electrophoresis and detected by staining or labeling. A molecular marker is a nucleotide sequence corresponding to a particular physical location in the genome. Therefore, molecular marker is a polypeptide or piece of DNA with easily identified phenotype such that cells or individuals with different alleles are distinguishable. It can be a protein, isozyme, short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like minisatellites, whose inheritance can be monitored. Genetic markers represent genetic differences between individual organisms or species. They may or may not represent the target genes themselves, can act as 'signs' or 'flags' when it does not represent the gene. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus').New developments in genomic research have given access to an enormous amount of sequence information as well as new insights on the function and

interaction of genes and the evolution of functional domains, chromosomes and genomes. In this context, functional and comparative genomics can help in comparative genetic mapping and linkage analysis of useful horticultural traits.

Application of molecular markers in vegetable crops:

Molecular markers have found application in every branch of plant biology from development of genetic linkage maps to marker-assisted selection to population genetics. The suitability of markers for the research purpose differs based on the purpose for which they are being employed. The various applications of molecular markers in plant biology are discussed in brief. The first reported case of MAS was in fact the association studies between blood group and production characteristics in Danish cattle breeds (Neimann-Sorenson and Robertson 1961). This was followed by a series of well-known and often cited papers on MAS related aspects such as linkage mapping, QTL analysis, etc., in plants (Tanksley and Rick 1980; Soller and Beckman 1983; Paterson et al. 1988). Notable among these studies are the selection studies involving isozyme markers in tomato (Tanksley et al. 1981) and maize (Stuber 1982), which is considered as the first real case of MAS in plants.Presently, molecular markers are used widely in improvement programme of vegetable crops. The major application of molecular markets in improvement of vegetable crops is as follows:

Characterization, Fingerprinting and Genetic Diversity

Genetic fingerprinting is the unambiguous identification of an individual (based on the presence or absence of alleles at different markers) or a population (based on frequencies of alleles of the markers). This is an absolute measure and does not change depending on other individuals or populations under study. Genetic diversity is a study undertaken to classify an individual or population compared to other individuals or populations. This is a relative measure, as the distance between any pair of entries in the study is greater or lesser depending on all pairwise comparisons that can be made in the study.

Molecular characterization can quantify and partition variation among genebank holding. The assessment of genetic identity, relatedness, and structure among entries and collections becomes a priority to characterize and conserve crop genetic resources. Phippen et al. (1997) tested 14 phenotypically uniform accessions of Brassica oleracea var. capitata (cabbage) with nine RAPD primers. These amplifications generated 110 fragments, of which 80 were polymorphic ranging in size from 370 to 1720 bp those were sufficient to distinguish between all 14 accessions. Molecular markers provides gene bank curators with additional sources of information to better plan and organize collection holdings and use finite financial support in a more effective manner. Thirteen SSRs were used (Tonguc and Griffiths 2004) to assess genetic similarity between 54 B. oleracea cultivars, belonging to 3 groups (cabbage, cauliflower, and broccoli). The varietal group with the narrowest genetic variation in the study was cauliflower (B. oleracea var. botrytis) followed by broccoli (B. oleracea var. italica) and cabbage (B. oleracea var. capitata) groups.

Phylogenetic relationship

In biology, phylogenetic is the study of evolutionary relatedness among various groups of organisms (e.g., species, populations), which is discovered through molecular sequencing data and morphological data matrices

Hybridity Test

A fast and accurate genetic purity test of F_1 hybrid plants is essential for seed production and accelerating advanced breeding generations in breeding programmes. DNA technology has great potential for enhancing purity assessment of hybrids.

Development of Genetic Linkage Maps

Linkage maps are constructed by ordering markers indicating the relative genetic distances (cM) between them, and assigning them to linkage groups on the basis of recombination values from all their pairwise combinations. They act as signposts in order to map genes governing useful economic traits. The basis and methodology for construction of linkage maps and their utility have been reviewed by Staub et al. 1996. Saturated linkage maps are available in rice, maize, wheat, barley, tomato, soybean, sunflower, etc.

Comparative Mapping

Comparative mapping refers to the prediction of linkage relationships in closely related/ distant taxa by using a common set of molecular markers. cDNA clones are most informative since they are sufficiently well conserved across species/ genera. Establishment of homologous regions in different crop specieshelps in studying their evolution. eg., In case of tomato and pepper, synteny was is preserved in nine chromosomes while paracentric inversions are observed in remaining three chromosomes.

Tagging of genes: The advent of molecular markers has enabled tagging of genes governing agronomically important traits such as disease and pest resistance in crop plants. They help in improving the efficiency of conventional plant breeding by carrying out indirect selection of the trait of interest through selection for molecular loci linked to that trait. These linked markers are highly useful in convergence breeding and breeding efforts aimed at developing more durable forms of resistance by pyramiding of two or more genes into an agronomically superior variety.

Map based Cloning/ Positional Cloning: Map based cloning is based on the identification of tightly linked markers on either side of the gene. Genomic libraries of large fragments can be screened with these linked markers so as to pinpoint the clones containing gene of interest (Tanskley et al. 1995). Chromosome walking is performed to identify/develop markers between the gene of interest and the identified linked marker. Further, these markers are used to fine-map the gene of interest and finally clone the target gene. Several genes have been cloned and functionally characterized. Some of the cloned genes are, fatty acid desaturase gene fad3 & RPS2 in Arabidopsis; Pto gene in tomato.

Marker Assisted Backcross Breeding: Molecular markers have proven very useful in accelerating backcross breeding programmes in space as well as time. DNA markers offer three distinct advantages in a backcross breeding program namely:

(i) Selection for the target gene
(ii) Selection for higher recurrent parent genome recovery
(iii) Selection against linkage drag:

Marker Assisted Selection (MAS)

MAS may greatly increase the efficiency and effectiveness in plant breeding compared to conventional breeding methods. Once markers that are tightly linked to genes or QTLs of interest have been identified, prior to field evaluation of large numbers of plants, breeders may use specific DNA marker alleles as a diagnostic tool to identify plants carrying the genes or QTLs (Young 1996).

Steps in MAS

Marker-assisted selection (MAS) is a method whereby a phenotype is selected on the genotype of a marker. However, the markers identified in preliminary genetic mapping studies are seldom suitable for marker-assisted selection without further testing and possibly further development. Markers that are not adequately tested before use in MAS programs may not be reliable for predicting phenotype, and will therefore be useless. Generally, the steps required for the development of markers for use in MAS includes: high resolution mapping, validation of markers and possibly marker conversion.

Validation of markers

Generally, markers should be validated by testing their effectiveness in determining the target phenotype in independent populations and different genetic backgrounds, which is referred to as marker validation. In other words, marker validation involves testing the reliability of markers to predict phenotype. This indicates whether or not a marker could be used in routine screening for MAS (Ogbonnaya et al. 2001; Sharp et al. 2001). Markers should also be validated by testing for the presence of the marker on a range of cultivars and other important genotypes (Sharp et al. 2001). Some studies have warned of the danger of assuming that marker-QTL linkages will remain in different genetic backgrounds or in different testing environments, especially for complex traits such as yield (Reyna and Sneller 2001). Even when a single gene controls a particular trait, there is no guarantee that DNA markers identified in one population will be useful in different populations, especially when the populations originate from distantly related germplasm (Yu et al. 2000). For markers to be most useful in breeding programs, they should reveal polymorphism in different populations derived from a wide range of different parental genotypes (Langridge et al. 2001).

Selection of QTLs for MAS

Like that of other crops, several horticultural traits in vegetables are also controlled by quantitative trait loci (QTLs). The goal of QTL mapping is to dissect the complex inheritance of quantitative traits into Mendelian-like factors amenable to selection through the analysis of the flanking molecular markers. These markers can then be used in molecular breeding and to clone

the genes controlling the QTLs. Although any segregating population can be used for RIL mapping, use of RILs has certain advantages. RILs are near homozygous, which allows multiple replicates to assess phenotypic values, reducing the environmental effects and increasing the power and accuracy to detect QTL. Once QTLs are identified, they can be introgressed to elite germplasm through MAS, much like monogenic traits. In cucumber and melons number QTLs have been mapped and their use in MAS is in progress. Many horticultural traits, including yield are under polygenic control with considerable environmental influence and genotype by environment interaction on trait expression.

Role of in vitro based tools

The biotechnology-based tools are used for mass propagation of disease-free planting material in number of horticultural crops. The modern biotechnological approaches such as embryo rescue, somatic hybridization and doubled haploids (DHs) have tremendous potentials to enhance the efficiency of traditional breeding and can greatly enhanced the precision in crop improvement. Further, the marker-assisted selection allows for a faster and more targeted development of improved genotypes. Such markers provide new research tools which can assist in the conservation and characterization of biodiversity.

Micropropagation:

Tissue culture is a technique of in vitro growing and multiplying plant tissues on artificial media under aseptic and controlled environmental conditions. The fact that a whole plant can be regenerated from a single cell, explant, or organ makes tissue culture a valuable procedure for proliferating genetically identical material. In vitro tissue culture techniques can accelerate breeding of vegetable crops, especially when applied to the maintenance of desirable parental lines of F_1 hybrids (Cristea et al., 2009). The F_1 hybrids are highly popular in vegetable crops. However, in order to maintain the genetic purity of hybrid seeds, self-pollination of the seed producing plant should be prevented. Micropropagation of these F_1 hybrids eliminates the need for manual emasculation for sterility or pollination and enables rapid multiplication. This technique can be further utilised for maintenance of male sterile lines either controlled by recessive genes (tomato, muskmelon, chilli) or dominant genes (cabbage); maintenance of self-incompatible and cytoplasmic male sterile lines in cole crops and maintenance of hybrids as such. In cole crops, cytoplasmic male sterility (CMS) system is commercially employed for development of F_1 hybrids. During maintenance, the CMS (A) lines are crossed with the maintainer (B) lines and the B line is maintained by self-pollination. However, maintenance of the B lines through self-pollination results in several adverse effects such as reduced vigour, reduced curd size, and deformed curds, due to inbreeding depression. As a result, the vigour of CMS lines reduced significantly during the process of maintenance. Hence, in vitro maintenance of parental lines through tissue culture can greatly enhance the production high purity F_1 hybrid seed production. It will also eliminate labour intensive bud pollination for maintenance of parental line (Bhatia et al., 2015).

Meristem culture

Garlic and shallot, which are propagated vegetatively, are known to harbour a number of viruses. Some of these viruses are latent, i.e., cause no visible symptoms, whereas others reduce yield considerably. It is possible to eliminate viruses in garlic and shallot using meristem culture technique. The dark-green "island" areas of the growing point (meristem) are either free of virus or contain virus at a very low concentrations. By taking 0.3 mm meristems under aseptic conditions and culturing them on a suitable medium, virus-free garlic and shallot plants can be obtained. In conjunction with the vigorous virus testing procedures, garlic and shallot lines have been have been produced which are free from onion yellow dwarf virus, garlic common latent virus, shallot latent virus, and leek yellow strip virus. This technique also facilitates international exchange of disease-free germplasm.

Synthetic Seed technology: A synthetic or artificial seed has been defined as a somatic embryo encapsulated inside a coating and is considered to be analogous to a zygotic seed. There are several different types of synthetic seed: somatic embryos encapsulated in a water gel; dried and coated somatic embryos; dried and uncoated somatic embryos; somatic embryos suspended in a fluid carrier; and shoot buds encapsulated in a water gel. No large-scale system for producing such seeds has yet been developed, although pilot studies of moderate size, using somatic embryos encapsulated in a water gel. have been conducted in Japan with F_1 hybrids of celery and lettuce (Sanada et al. 1993) and asparagus (Mamiya & Sakamoto, 2000). More recently it has been reported that pharmaceutical type capsules can be used as coating system which act as a strong water soluble hull, covered on its inner surface by water-impermeable film of polyvinyl chloride, polyvinyl acetate and bentone as thickener to control nutrient supply and subsequent development of somatic embryos. The use of synthetic seeds as an improvement on more traditional micropropagation protocols in vegetatively propagated crops may, in the long term, have a cost saving, as the labour intensive step of transferring plants from in vitro to soil/field conditions may be overcome. Other applications include the maintenance of male sterile lines, the maintenance of parental lines for hybrid crop production, and the preservation and multiplication of elite genotypes of woody plants that have long juvenile developmental phases. However, before the widespread application of this technology, somaclonal variation will have to be minimized, large-scale production of high quality embryos must be perfected in the species of interest, and the protocols will have to be made cost-effective compared with existing seed or micropropagation technologies. Synthetic seed technology has a great potential to multiply true-to-type hybrids. By propagating male sterile and male fertile or self-sterile parental lines through synthetic seeds, a pollination control system can be introduced in plant population. A small number of hybrids can be produced by hand pollination and subsequently mass produced by artificial seed technology, thus making it possible to exploit the hybrid vigour in crop where the production of hybrid is difficult.

In vitro pollination and fertilization:

The techniques of in vitro pollination and fertilization have been successfully used in interspecific and intergeneric hybridization to overcome the pre-fertilization barriers. In in vitro

pollination pollen is directly applied to the stigma of cultured ovaries or directly on to ovules cultured with or without placenta. To overcome the pre-zygotic barriers that hinder the growth of the pollen on the stigma or style, a part of the stigma or style may be cut and the pollen grain may be placed on the cut surface of the ovary or transferred through a hole in the ovary wall (intra ovarian pollination). Another approach to overcome the barrier to pollen tube growth is direct pollination of cultured ovules (in vitro ovular pollination). Pollination of ovules along with placenta is termed as in vitro placental pollination. In vitro fertilization (IVF) refers to fusion of isolated male and female gametes in vitro to form zygote, which ultimately would give rise to normal individual. IVF has been used to facilitate both interspecific and intergeneric crosses, to overcome physiological-based self-incompatibility and to produce hybrids. A wide range of plant species has been recovered through IVF via pollination of pistils and self- and cross-pollination of ovules.

Embryo rescue

Sexual hybridization is a valuable tool for the conventional plant breeding to improve cultivated crops. However, many desirable traits cannot be transmitted via conventional methods of genetic manipulation. The technique of conventional hybridization is limited to only very closely related species and cannot be used to transfer the traits from distantly related species as well as in sexually incompatible species. Wide hybridization is one of the oldest breeding techniques that contributed enormously to the development of modern plant cultivars. Interspecific and intergeneric crosses are very effective in terms of broadening genetic variability and introgression of desirable traits from wild species.

Protoplast fusion

Protoplast culture and somatic cell hybridization involving the fusion of protoplasts from different plant species is one of the important approaches for combining characteristics from otherwise sexually incompatible species. The prezygotic barriers such as gametic incompatibities during wide hybridization can be overcome by the techniques of somatic hybridization. This is a non-conventional approach of transfer of gene/genes, involving fusion between isolated protoplasts from unrelated species/genera under in vitro condition and subsequent development of their product (heterokaryon) to a hybrid plant. During sexual crossings, the plasmid and mitochondrial genomes are inherited maternally; however, the protoplast fusion technique combines the nucleus and cytoplasm of both the parents in the hybrid cell. Further, cybrids (cytoplasmic hybrids) and organelle recombinants, not possible through conventional methods, can also be developed. Cybrids in contrast to conventional hybrids possesses the nucleus genome from only one parent but cytoplasmic genes of both the parents. Recombinant organellar genomes, especially of mitochondria, are generated in somatic hybrids and cybrids. In comparison with sexual hybridization, somatic cell fusion is a much more powerful method for increasing cytoplasmic diversity in a population as it is possible to transfer chloroplasts and mitochondria in a single step, even between incongruent species, and to obtain novel combinations of nucleus and cytoplasm organelles. In cybridization, heterozygosity of extra-chromosomal material can be obtained, which has direct application in

plant breeding. The production of cytoplasmic hybrids (cybrids) is particularly attractive when one is interested in the transfer of cytoplasmic-encoded traits into a particular genetic background. In most of the crop species, any two protoplasts can be fused by chemical or physical means under in vitro conditions. However, production of unique somatic hybrid plants is limited by the ability to regenerate the fused product and sterility in the interspecific hybrids rather than the production of protoplasts.

Haploids

Haploids and doubled haploids are very important in plant breeding, enabling the time needed to produce homozygous lines to be shortened compared with conventional breeding. Plants with gametophytic chromosome number in their sporophyte (whether diploid or polyploid) are referred to as haploids. Haploids of diploid species are termed as monoploids since they have a single copy of one genome. In contrast, haploids of tetraploid species e.g. potato, have single copy of two genomes and are referred as dihaploids. Doubled haploids can be produced in vivo or in vitro. Haploid embryos are produced in vivo by parthenogenesis, pseudogamy, or chromosome elimination after wide crossing. The haploid embryo is rescued, cultured, and chromosome-doubling produces doubled haploids. The in vitro methods include gynogenesis (ovary and flower culture) and androgenesis (anther and microspore culture). Haploid cells occur naturally in the gametophytic phases of higher plants in their ovules and pollen. By manipulating the gametic cells, it is possible to produce embryos rather than mature pollen grains or ovules. Androgenesis is the most preferred method for haploid production. In general haploids are much weaker than the normal plants. In most cases they are sterile and difficult to maintain, therefore, chromosome number of haploids is doubled to produce doubled haploids (DH), which have the normal somatic (2n) chromosome complement of the species and are fully fertile. By induced or spontaneous chromosome doubling, a completely homozygous doubled haploid plant can be produced. Conventional inbreeding procedures take six generations to achieve completely homozygous condition, whereas doubled haploidy reduces it to one step. Hence, it is of a great importance in plant breeding. The first report of the haploid plant was published by Blakeslee et al. (1922) in Datura stramonium. Subsequently, haploids were reported in many other species. Guha and Maheswari (1964) developed an anther culture technique for the production of haploids in the laboratory. Doubled haploid methodologies have now been applied to over 250 species.

Haploid plants are of interest to plant breeders because they allow the expression of simple recessive genetic traits or mutated recessive genes and because doubled haploids can be used immediately as homozygous breeding lines. The haploids and doubled haploids have wide application in genetic studies, plant breeding and basic research. The DH lines are also very useful for construction of genetic maps, locate genes of agronomic and economic importance, identify markers for trait selection, accelerate crop improvement programs and increase plant breeding efficiency (Forster and Thomas 2005; Chan and Pauls 2007; Ferrie and Caswell 2011; Bhatia et al., 2016). The techniques used for induction of haploids in different vegetable crops are enlisted in **Table 4**

Somaclonal variation

In addition to the variants/mutants (cell lines and plants) obtained as a result of the application of a selective agent in the presence or absence of a mutagen, many variants have been obtained through the tissue-culture cycle itself. These somaclonal variants, which are dependent on the natural variation in a population of cells, may be genetic or epigenetic, and are usually observed in the regenerated plantlets. Somaclonal variation itself does not appear to be a simple phenomenon, and may reflect pre-existing cellular genetic differences or tissue culture- induced variability. The variation may be generated through several types of nuclear chromosomal re-arrangements and losses, gene amplification or de-amplification: nonreciprocal mitotic recombination events, transposable element activation, apparent point mutations, or re-activation of silent genes in multigene families, as well as alterations in maternally inherited characteristics. Many of the changes observed in plants regenerated invitro have potential agricultural and horticultural significance. These include alterations in plant pigmentation, seed yield, plant vigour and size, leaf and flower morphology, essential oils, fruit solids and disease tolerance or resistance. Such variations have been observed in many crops, including wheat, triticale, rice, oats, maize, sugar cane, alfalfa, tobacco, tomato, potato, oilseed rape and celery. The same types of variation obtained from somatic cells and protoplasts can also be obtained from gametic tissue. One of the major potential benefits of somaclonal variation is the creation of additional genetic variability in co adapted, agronomically useful cultivars, without the need to resort to hybridization. This method could be valuable if selection is possible in vitro, or if rapid plant-screening methods are available. It is believed that somaclonal variants can be enhanced for some characters during culture in vitro, including resistance to disease pathotoxins and herbicides and tolerance to environmental or chemical stress. However, at present few cultivars of any agronomically important crop have been produced through the exploitation of somaclonal variation

Other innovative tools for improvement of vegetable crops

Innovation in improvement technologies is necessary to meet the challenges of global changes such aspopulation growth and climate change. For increasing world population and the need to protect the environment, the limited resources of land and water have to be used more efficiently for crop production. Besides, based on statistics from FAO, food production must be doubled between 2000 and 2050 to ensure food and nutritional security to the growing population. Therefore, plants with useful traits for disease and insect pest resistance, herbicide and stress tolerance and improved product quality characteristics have to be developed. Below are the description of new innovative breeding techniques and their utility in improvement of crops for the future.

Grafting (on GM rootstock)

Grafting is a method whereby the above ground vegetative component of one plant (also known as the scion), is attached to a rooted lower component (also known as the rootstock) of another plant to produce a chimeric organism with improved cultivation characteristics.

Transgenesis, cisgenesis and a range of other techniques can be used to transform the rootstock and/or scion. If a GM scion is grafted onto a non- GM rootstock, then stems, leaves, flowers, seeds and fruits will be transgenic. When a non-GM scion is grafted onto a GM rootstock, leaves, stems, flowers, seeds and fruits would not carry the genetic modification with respect to changes in genomic DNA sequences. Transformation of the rootstock can be obtained using traditional techniques for plant transformation, e.g. Agrobacterium-mediated transformation and biolistic approaches. Using genetic modification, characteristics of a rootstock including rooting capacity or resistance to soil borne diseases, can be improved, resulting in a substantial increase in the yield of harvestable components such as fruit.

If gene silencing in rootstocks is an objective this can also be obtained through RNA interference (RNAi), a system of gene silencing that employs small RNA molecules. In grafted plants, the small RNAs can also move through the graft so that the silencing signal can affect gene expression in the scion. RNAi rootstocks may therefore be used to study the effects of transmissible RNAi-mediated control of gene expression.

Reverse Breeding

Reverse breeding is a method in which the order of events leading to the production of a hybrid plant variety is reversed. It facilitates the production of homozygous parental lines that, once hybridised, reconstitute the genetic composition of an elite heterozygous plant, without the need for back-crossing and selection.

The method of reverse breeding includes the following steps:

• Selection of an elite heterozygous line that has to be reproduced;

• Suppression of meiotic recombination in the elite heterozygous line through silencing of genes such as dmc1 and spo11 following plant transformation with transgenes encoding RNA interference (RNAi) sequences;

• Production of haploid microspores (immature pollen grains) from flowers of the resulting transgenic eliteheterozygous line;

• Use of doubled haploid (DH) technology to double the genome of the haploid microspores and to obtain homozygous cells;

• Culture of the microspores in order to obtain homozygous diploid plants;

• Selection of plant pairs (called parental lines) that do not contain the transgene and whose hybridisation would reconstitute the elite heterozygous line.

The reverse breeding technique makes use of transgenesis to suppress meiotic recombination. Insubsequent steps, only non-transgenic plants are selected. Therefore, the offspring of the selectedparental lines would genotypically reproduce the elite heterozygous plant and would not carry anyadditional genomic change.

CRISPR/Cas technology

More recently, a new class of genome-editing technology, i.e., the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated) system, has been developed. The principle of the CRISPR/Cas system was derived from a type II prokaryotic organism adaptive immune system. Since the first report in early 2013, this technology has been widely

applied in gene modification in both animals and plants, such as zebrafish, mice, human cells, Arabidopsis, tobacco, rice, wheat, and sweet orange. In plants, the modified traits include changing plant architecture, e.g., Miao et al. 2013 changed the tiller angle by modifying the LAZY1 gene in rice, and Shan et al. 2013 changed the plant colour to white by modifying the OsSPD gene in rice. The first and foremost advantage of CRISPR/Cas system is based on simple RNA/DNA hybrids that confer sequence specificity. Secondly, it can simultaneously introduce multiple gene disruptions, which allows researchers to edit multiple genes in one plant line through a singletransformation without the time consuming post-transgenic hybridization and screening processes.

Therefore, CRISPR/Cas method is considered to be the most efficient, least expensive, and most user-friendly among genome-editing technologies. Compared to conventional and GM crops, it is advantageous in four domains i.e. precision, speed, cost and regulation.

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National Seed Research & Training Centre
G. T. Road, Collectry Farm,
Varanasi- 221106 (U.P.)
Tel: 0542 2370222
mail: dir-nsrtc-up@nic.in Website: www.nsrtc.ni