Practical Manual

Commercial Plant Breeding

Course No. AES-395, Credit Hrs. 3(1+2)

Dr. Rakesh Choudhary

2020

Department of Genetics and Plant Breeding
College of Agriculture
Rani Lakshmi Bai Central Agricultural University
Jhansi – 284003

Syllabus AES-395, Credit Hrs. 3(1+2):

Floral biology in self- and cross-pollinated species, selfing and crossing techniques. Techniques of seed production in self- and cross-pollinated crops using A/B/R and two-line system. Learning techniques in hybrid seed production using male-sterility in field crops. Understanding the difficulties in hybrid seed production, Tools and techniques for optimizing hybrid seed production. Concept of rouging in seed production plot. Concept of line its multiplication and purification in hybrid seed production. Role of pollinators in hybrid seed production. Hybrid seed production techniques in sorghum, pearl millet, maize, rice, rapeseed-mustard, sunflower, castor, pigeon pea, cotton and vegetable crops. Sampling and analytical procedures for purity testing and detection of spurious seed. Seed drying and storage structure in quality seed management. Screening techniques during seed processing viz., grading and packaging. Visit to public private seed production and processing plants.

Name of Student .....

Roll No. .....

Batch .....

Session .....

Semester .....

Course Name : .....

Course No. : .....

Credit .....

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Date: Course Teacher

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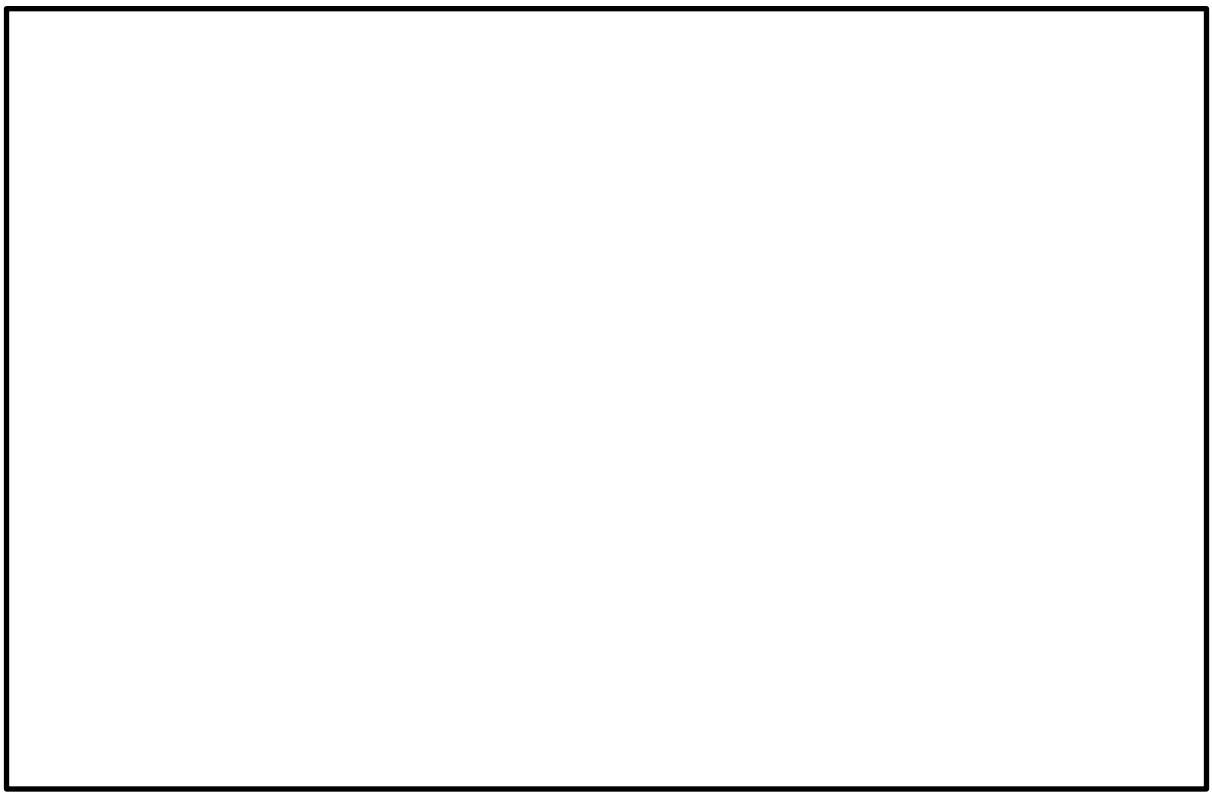
Practical No. 1

Objective: To study about floral biology of self-pollinated cereal crop species.

Exercise: Dissect the flowers given to you (Wheat, Rice and Barley) and sketch the diagram of inflorescence, single flower and separate floral parts.



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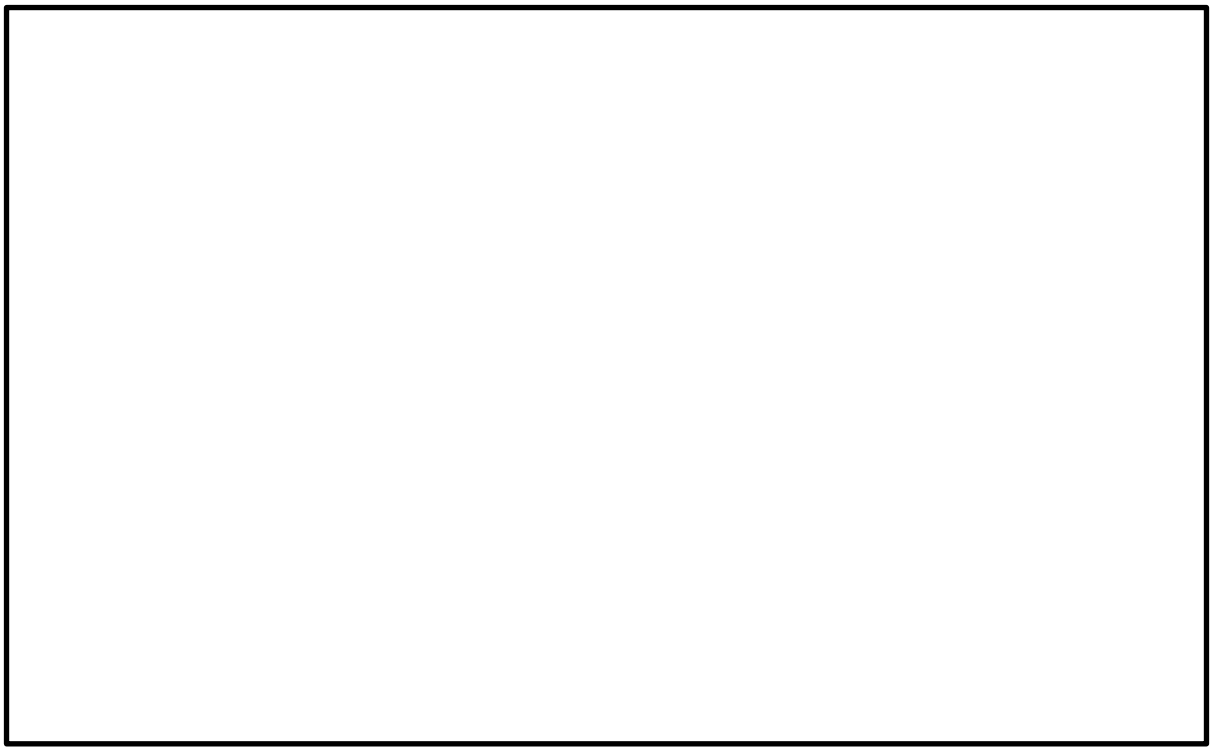

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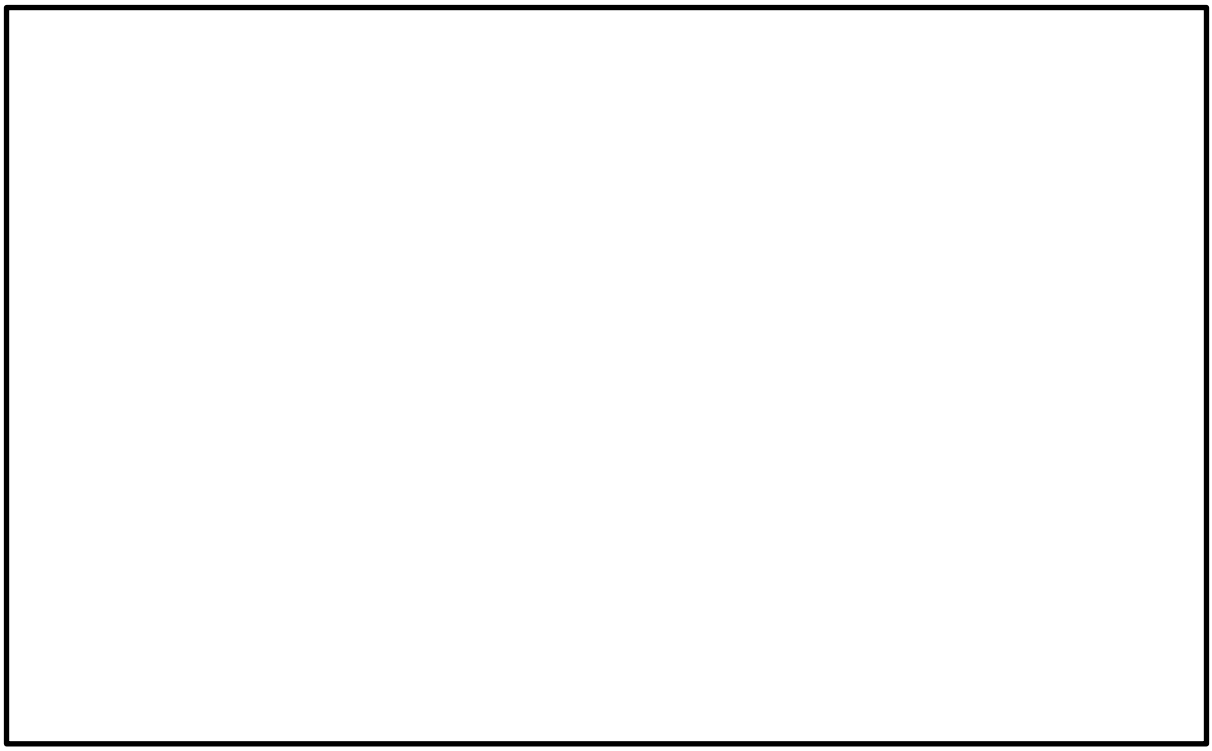

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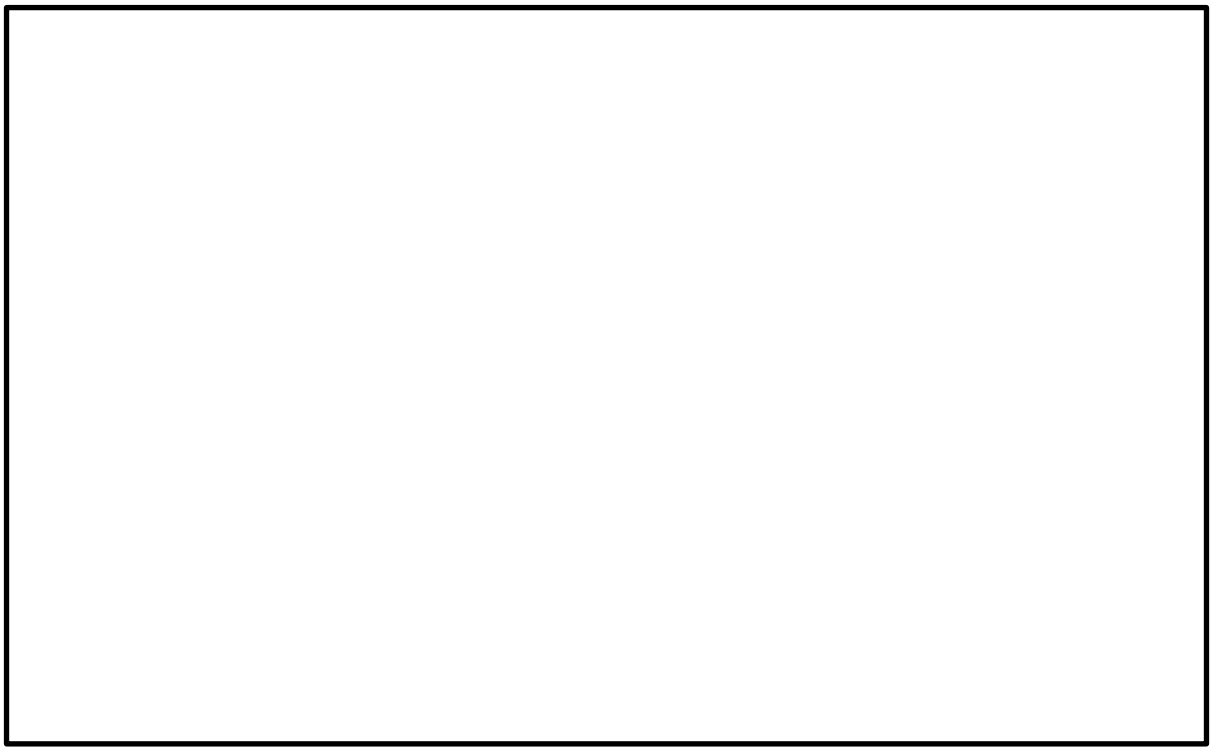
Practical No. 2

Objective: To study about floral biology of self-pollinated Millet crop species.

Exercise: Dissect the flowers given to you (Finger Millet, Kodo Millet and Barnyard Millet) and sketch the diagram of inflorescence, single flower and separate floral parts.



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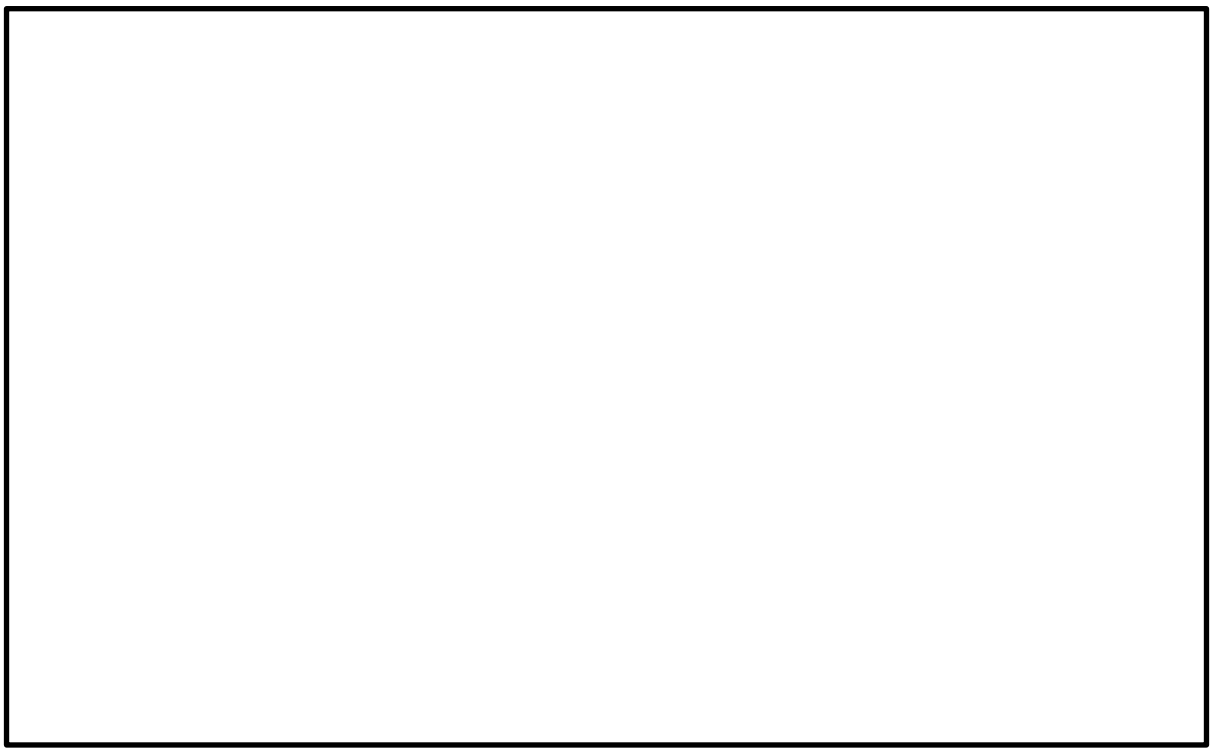

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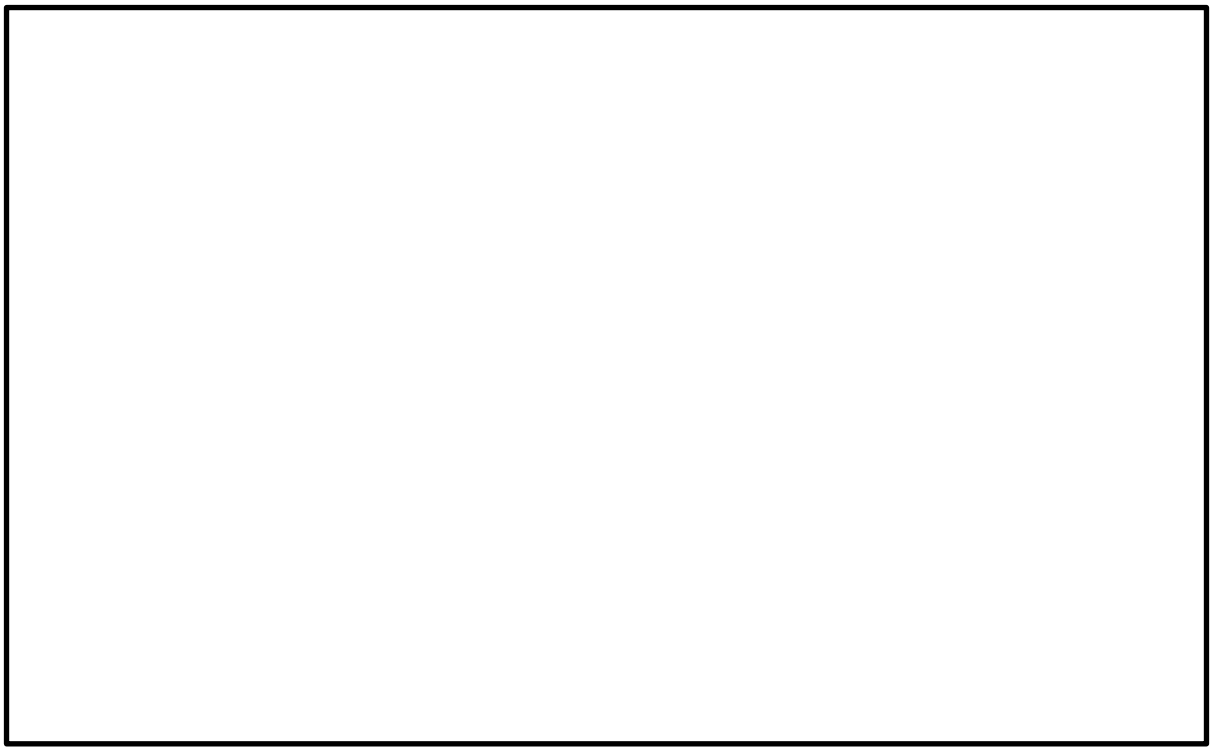

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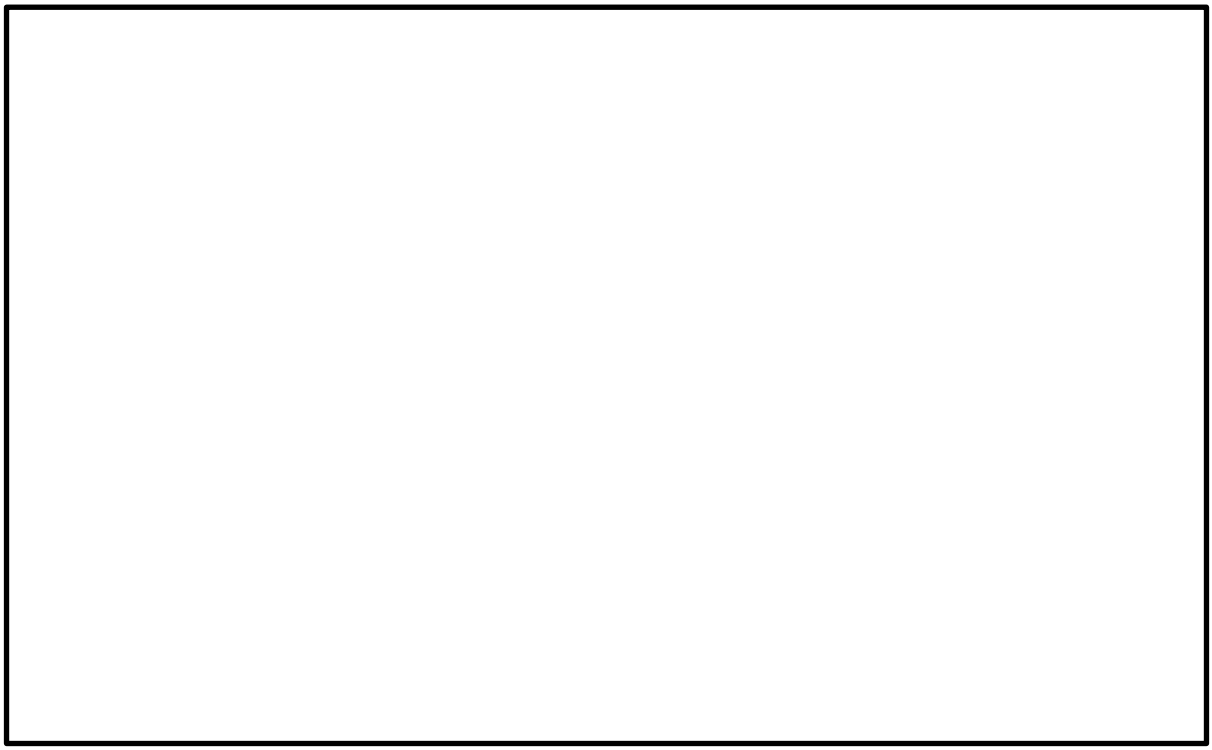
Practical No. 3

Objective: To study about floral biology of self-pollinated Pulse crop species.

Exercise: Dissect the flowers given to you (Chick pea, Green gram and Black gram) and sketch the diagram of inflorescence, single flower and separate floral parts.



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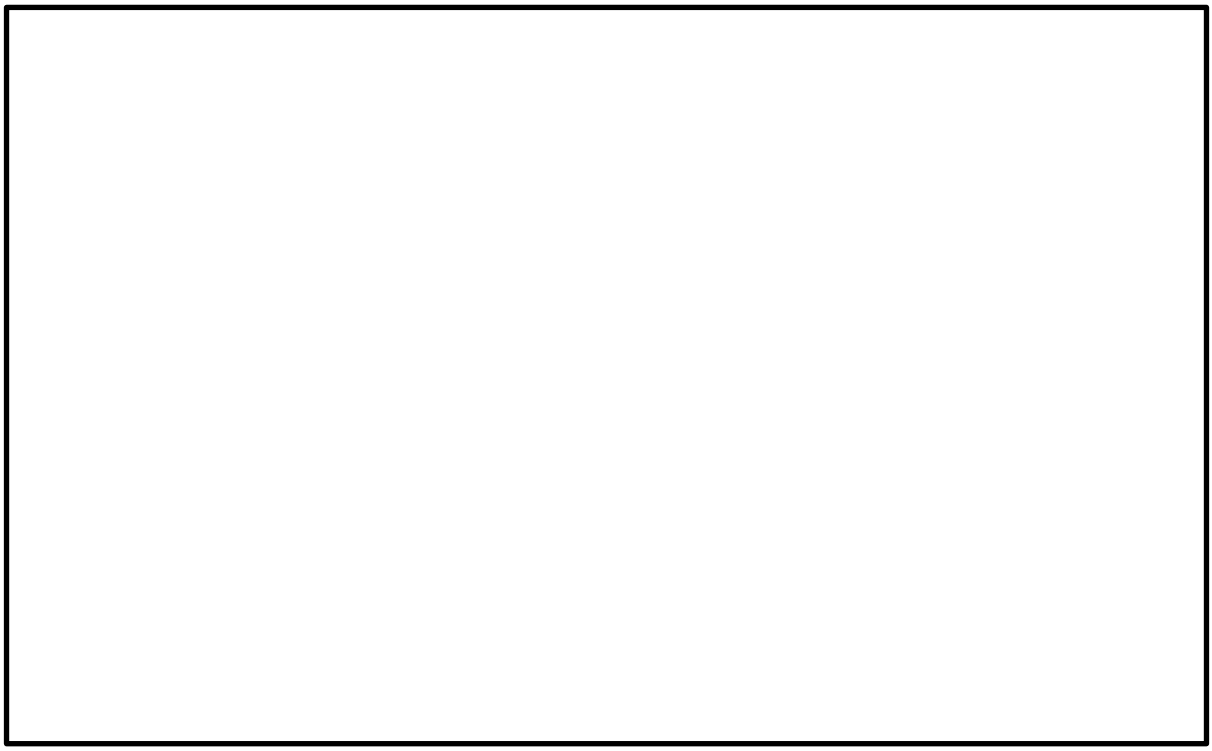

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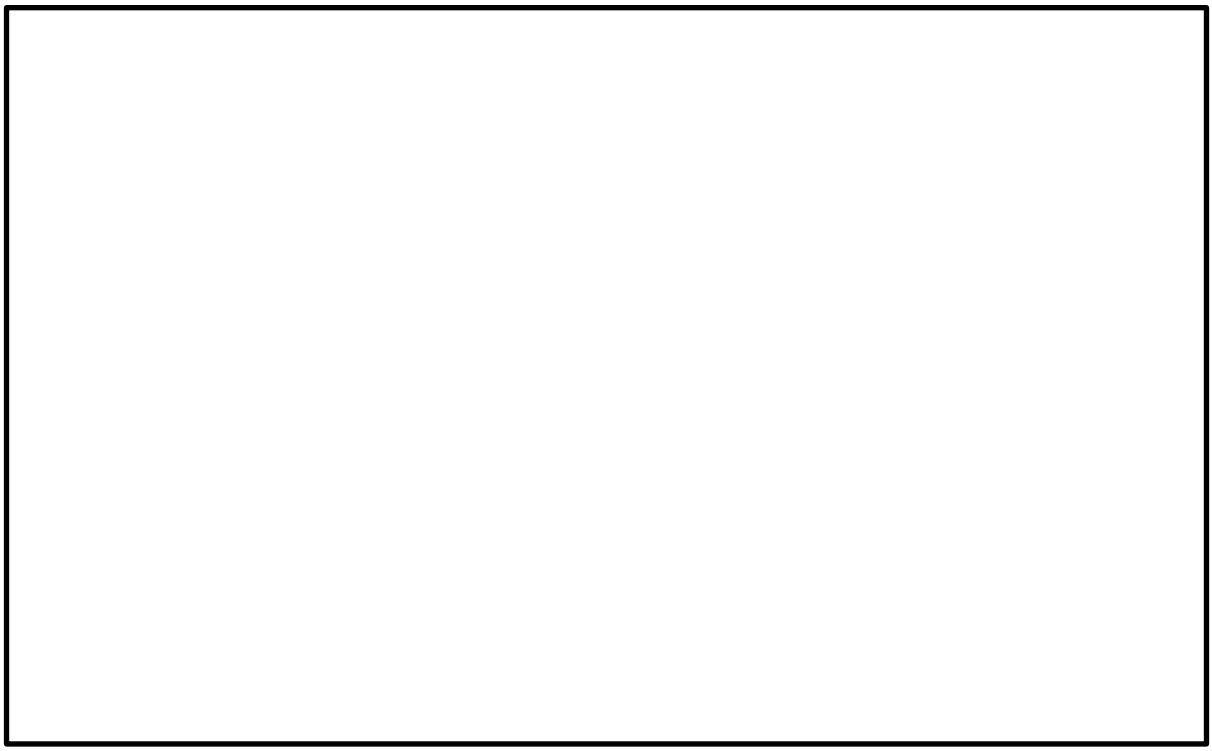

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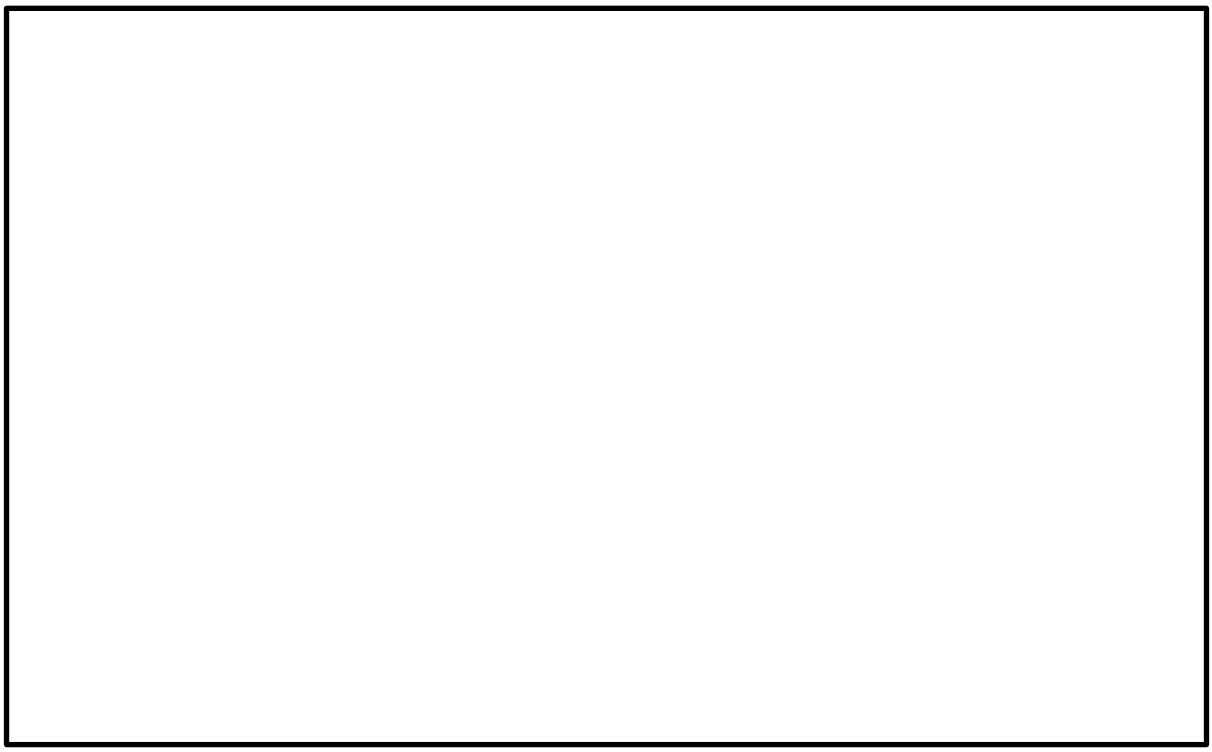
Practical No. 4

Objective: To study about floral biology of self-pollinated Pulse crop species.

Exercise: Dissect the flowers given to you (Field pea and Lentil) and sketch the diagram of inflorescence, single flower and separate floral parts.



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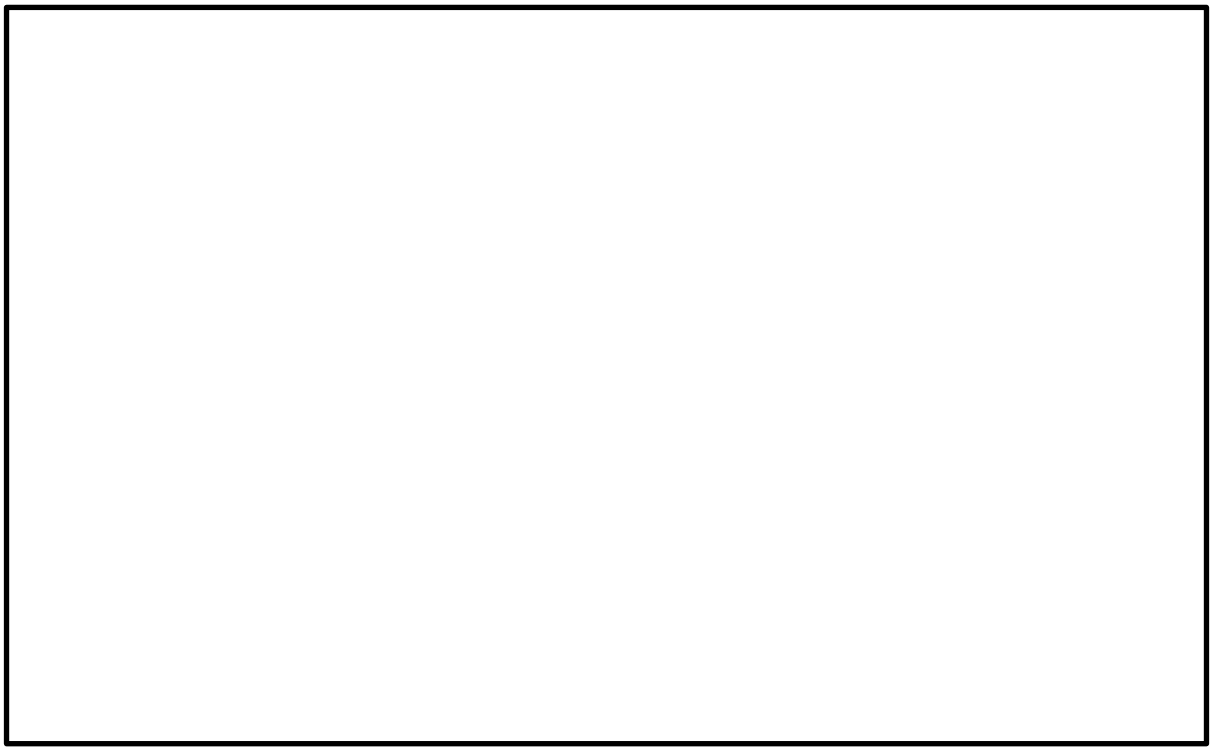

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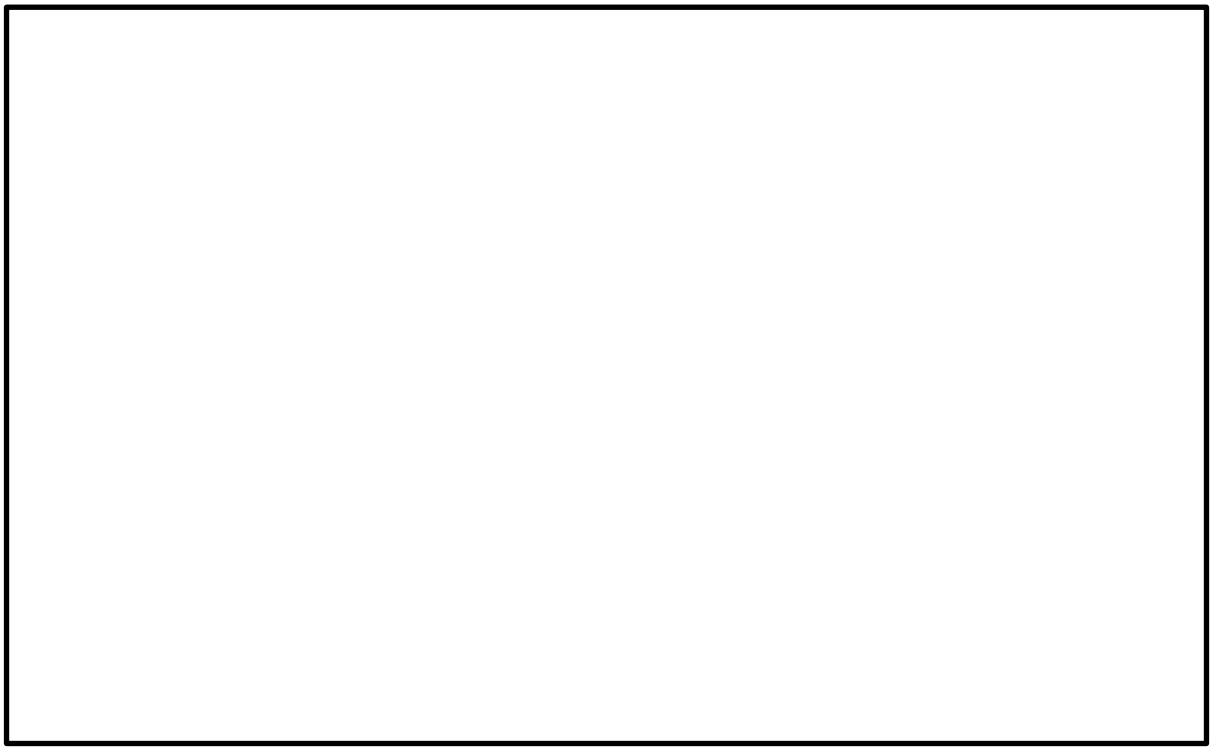
Practical No. 5

Objective: To study about floral biology of self-pollinated Oilseed crop species.

Exercise: Dissect the flowers given to you (Soybean, Sesame and Ground nut) and sketch the diagram of inflorescence, single flower and separate floral parts.



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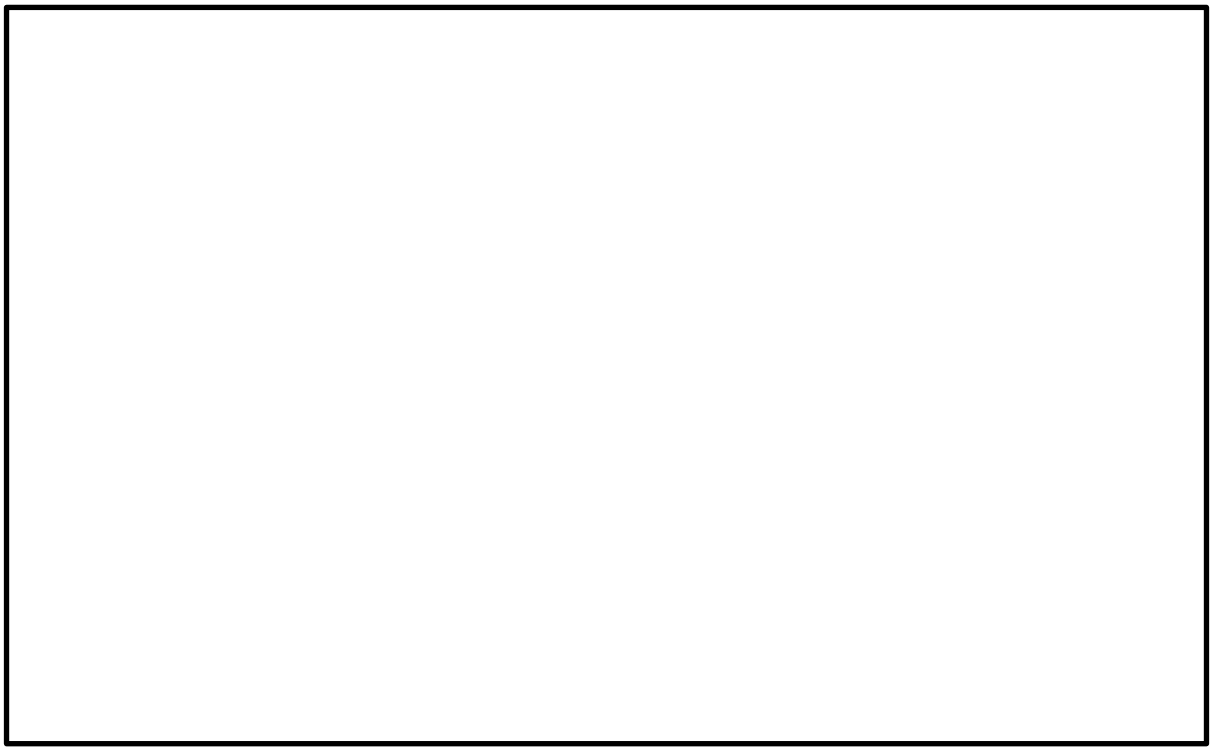

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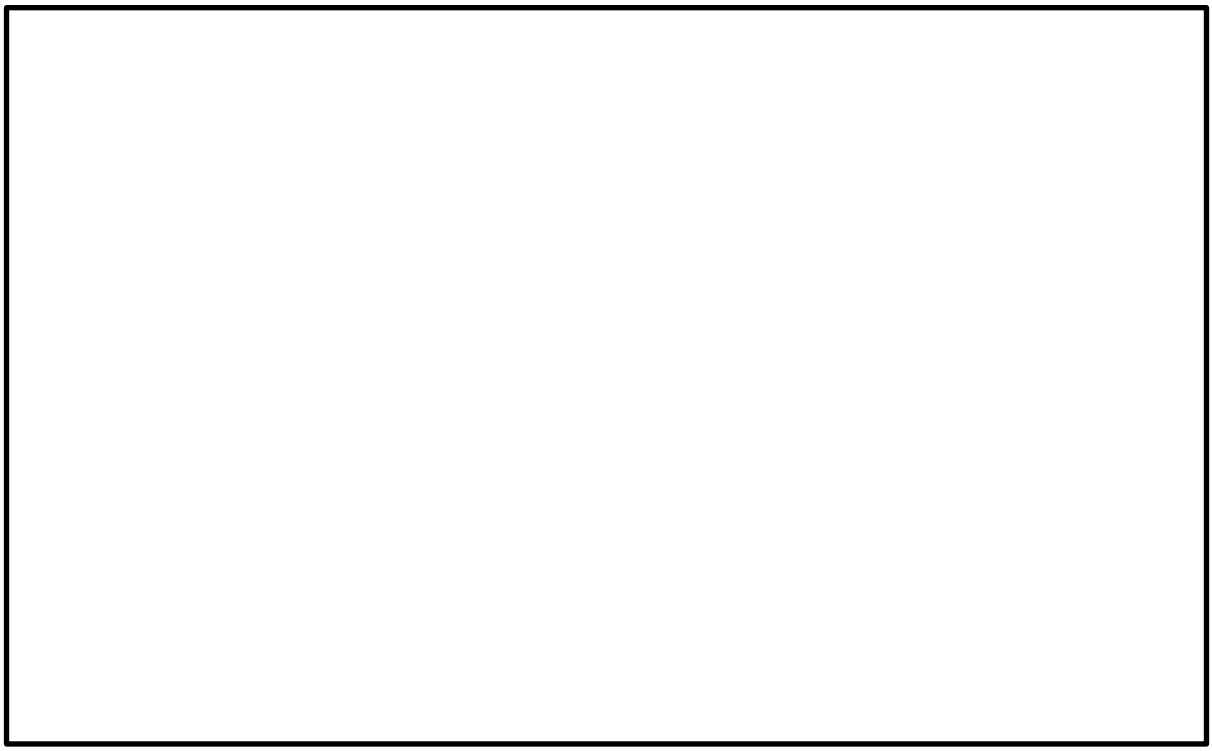

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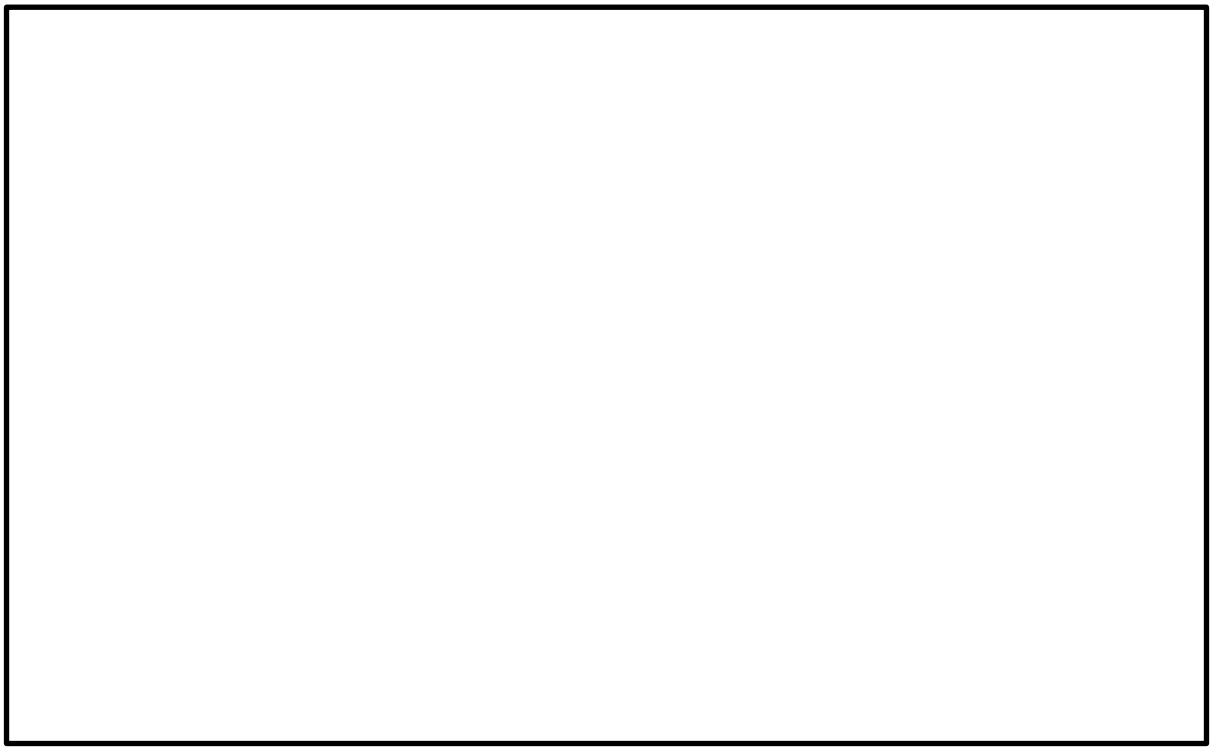
Practical No. 6

Objective: To study about floral biology of self-pollinated Oilseed crop species.

Exercise: Dissect the flowers given to you (Linseed and Rapeseed & Mustard) and sketch the diagram of inflorescence, single flower and separate floral parts.



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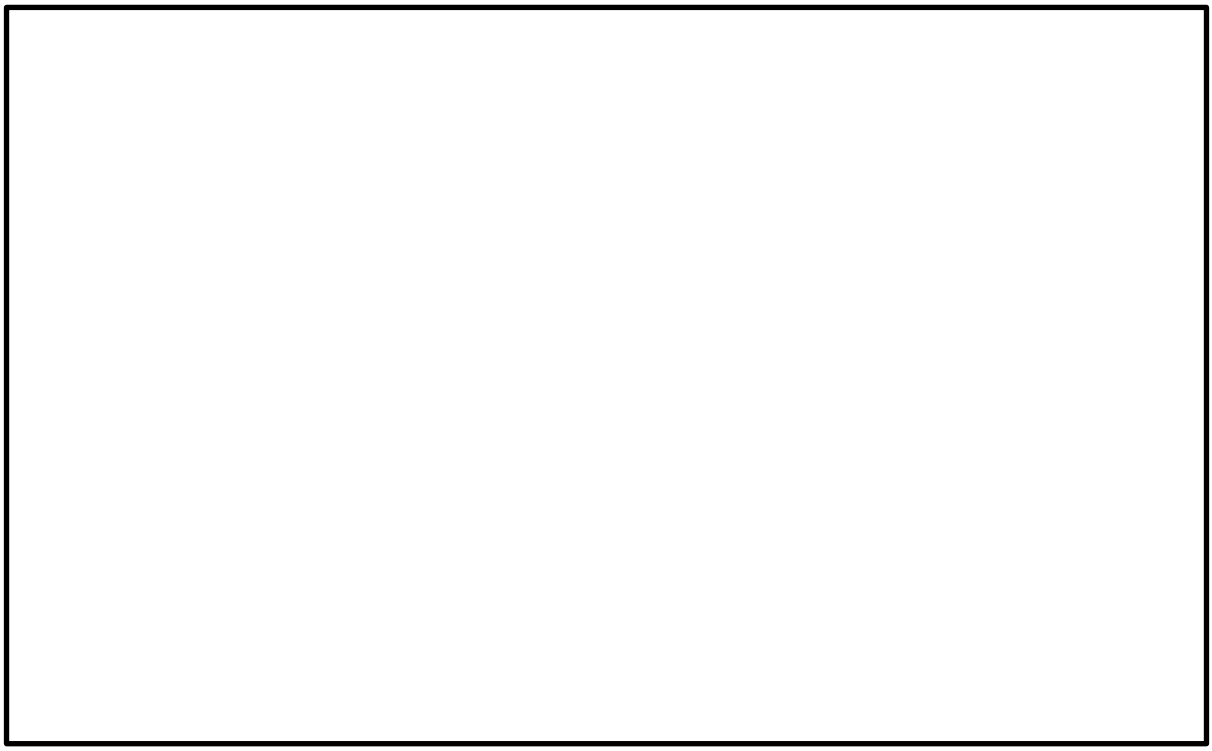

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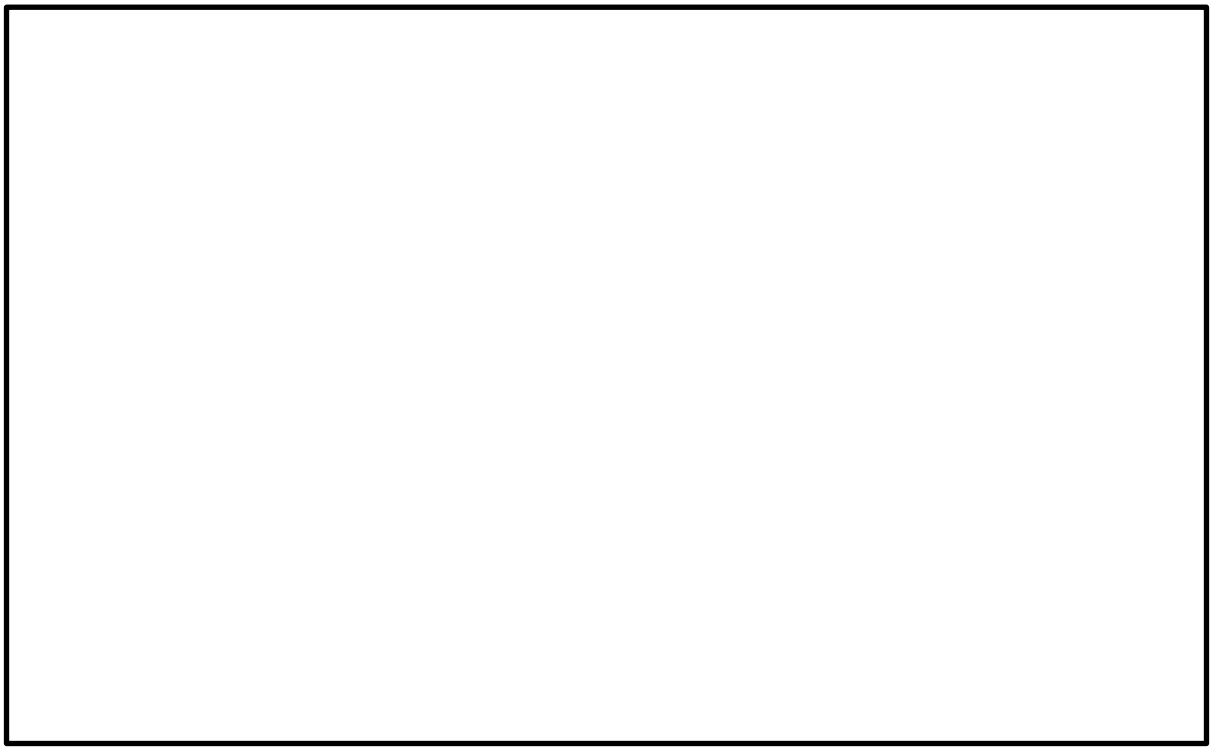
Practical No. 7

Objective: To study about floral biology of self-pollinated Vegetable crops.

Exercise: Dissect the flowers given to you (Tomato, okra and chilli) and sketch the diagram of inflorescence, single flower and separate floral parts.



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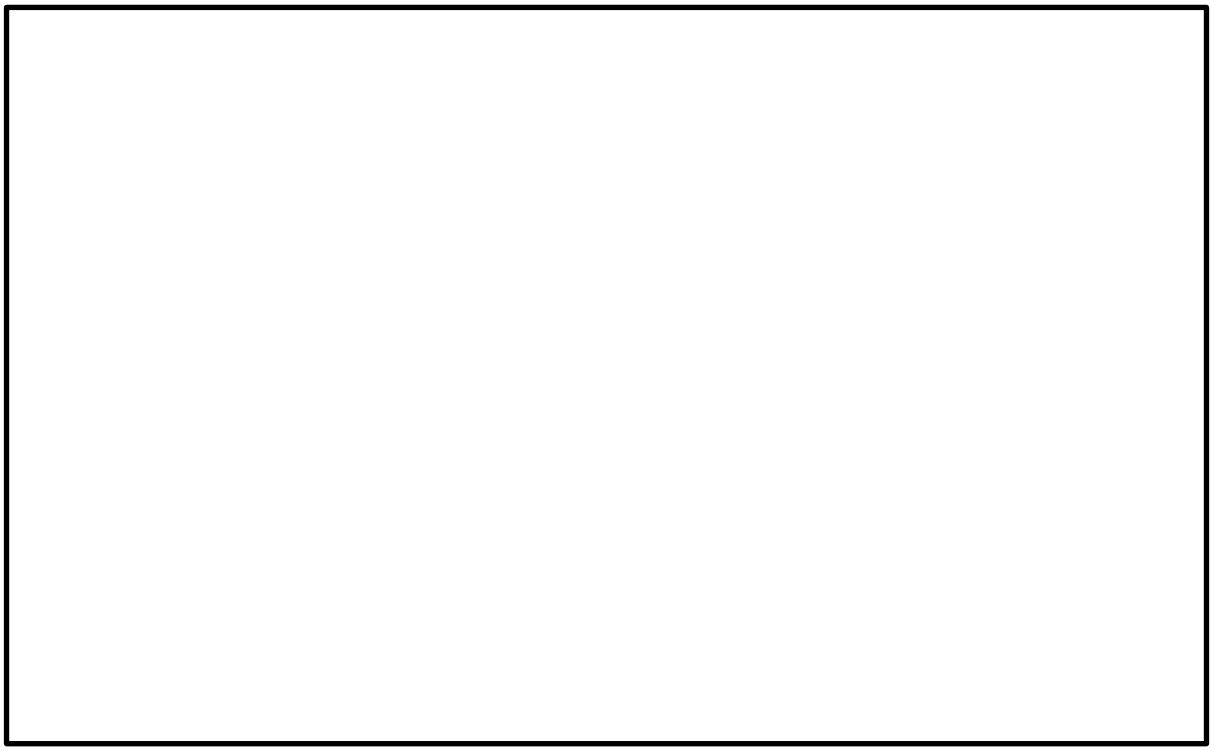

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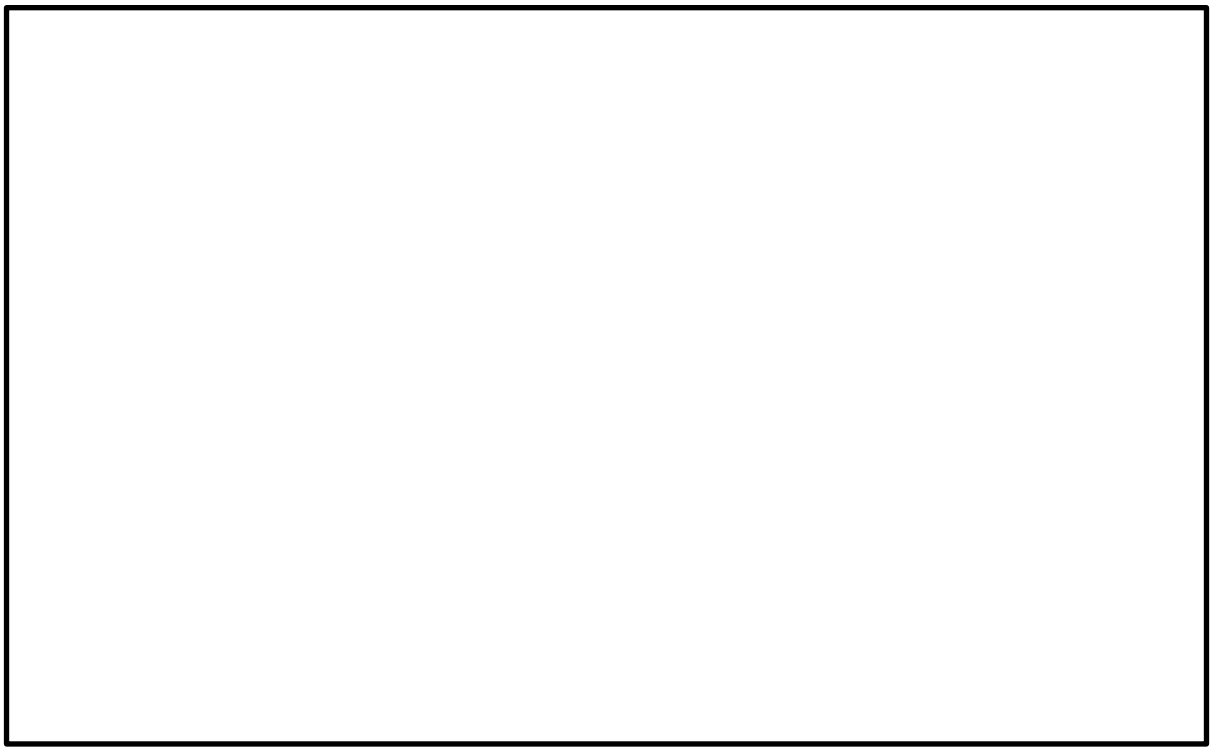

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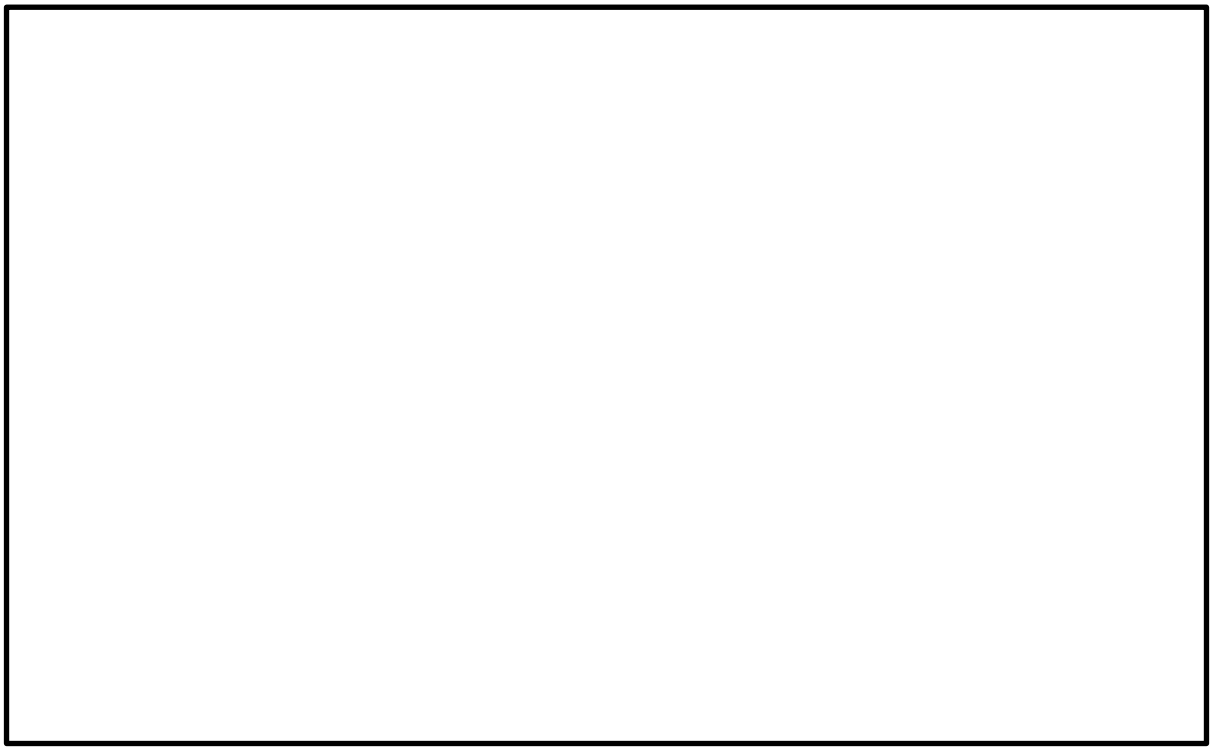
Practical No. 8

Objective: To study about floral biology of self-pollinated Vegetable crops.

Exercise: Dissect the flowers given to you (Potato and Brinjal) and sketch the diagram of inflorescence, single flower and separate floral parts.



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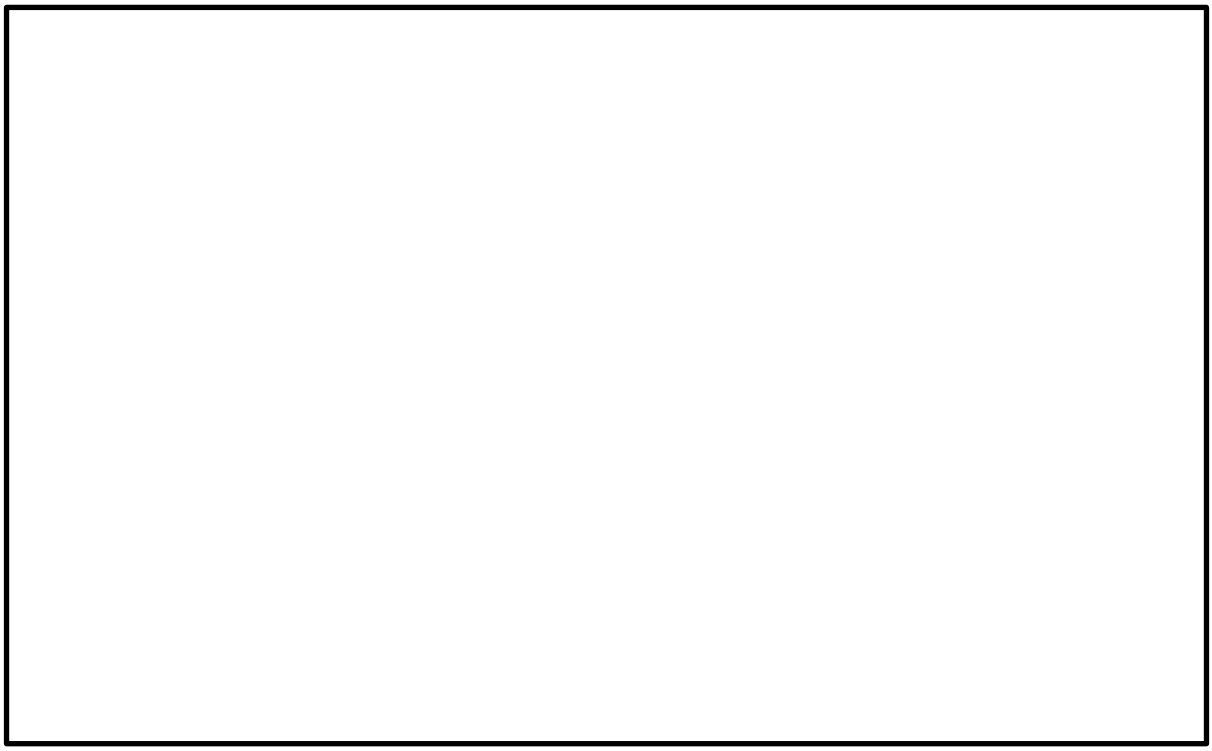

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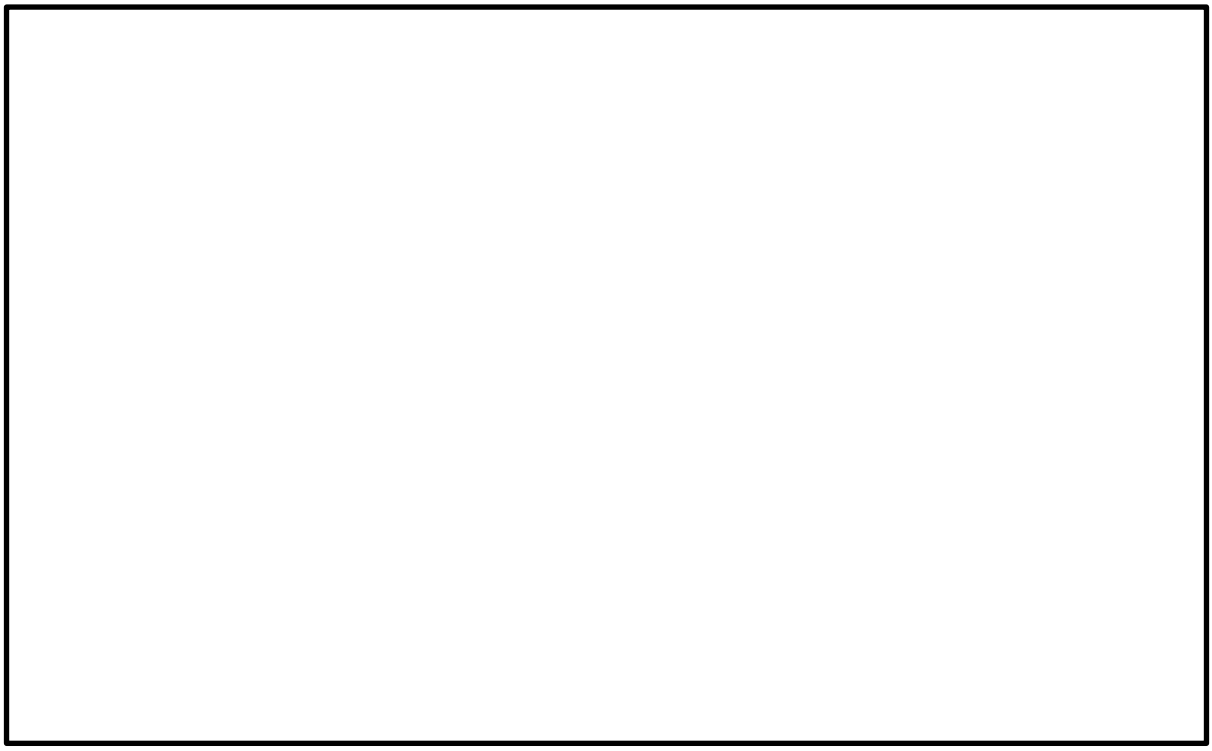
Practical No. 9

Objective: To study about floral biology of cross and often cross-pollinated cereals and millet crops.

Exercise: Dissect the flowers given to you (Maize, Pearl millet and Sorghum) and sketch the diagram of inflorescence, single flower and separate floral parts.



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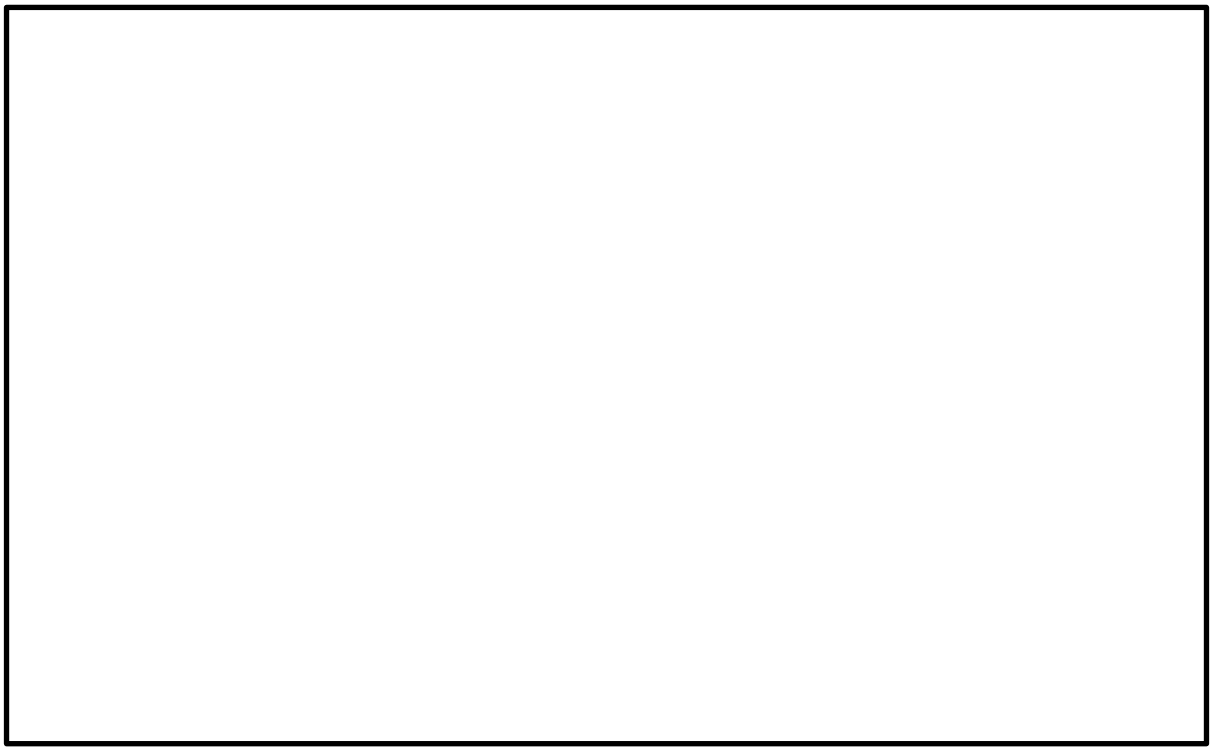

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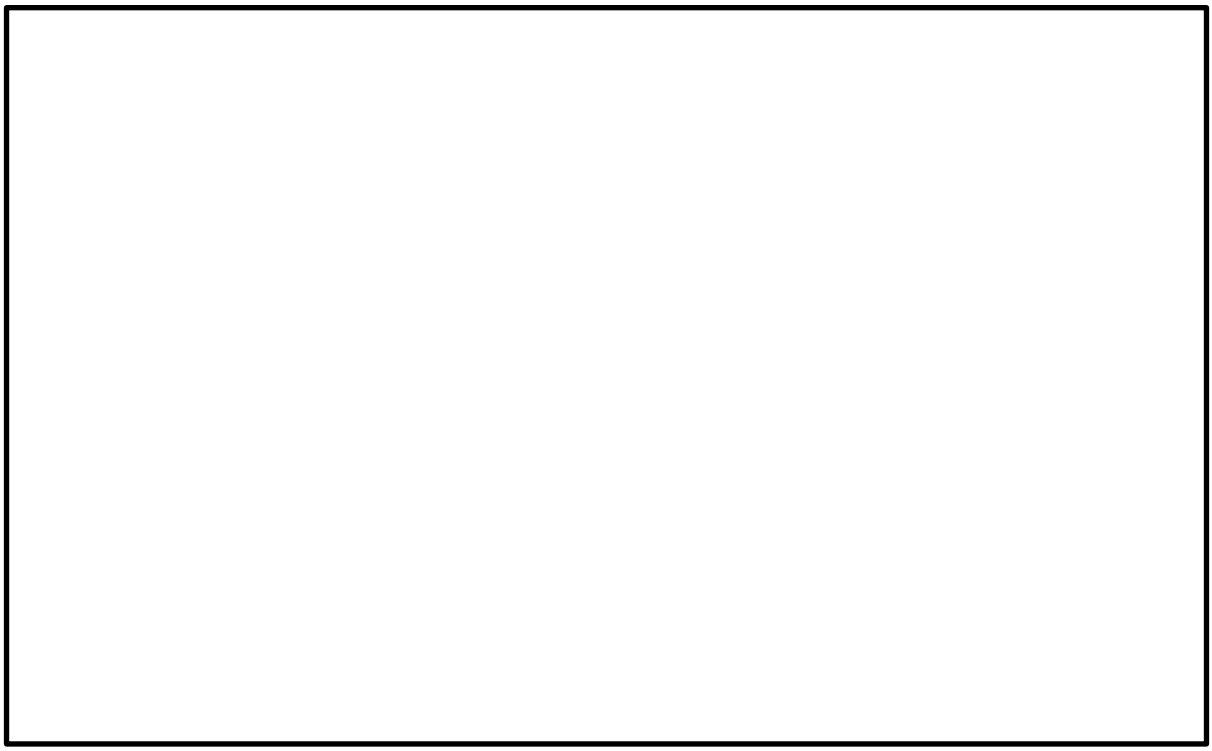

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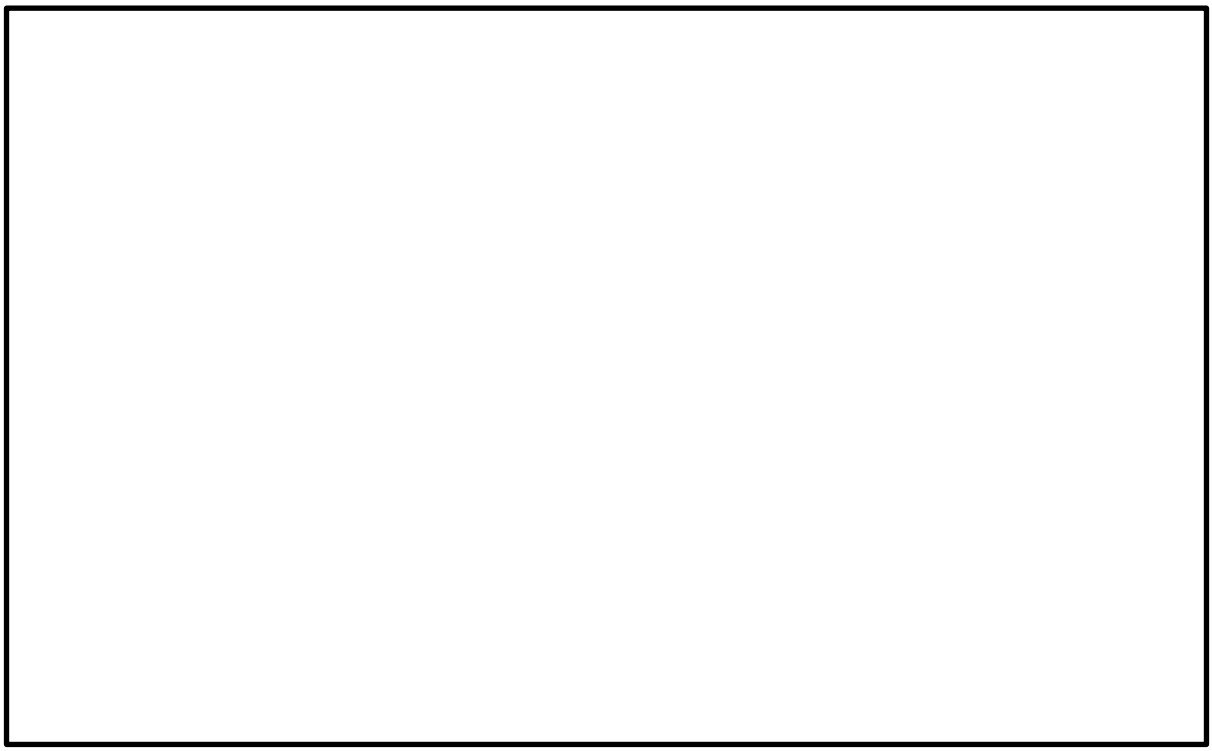
Practical No. 10

Objective: To study about floral biology of cross and often cross-pollinated Pulses and Oilseed crop species.

Exercise: Dissect the flowers given to you (Pigeon pea, Sunflower and Safflower) and sketch the diagram of inflorescence, single flower and separate floral parts.



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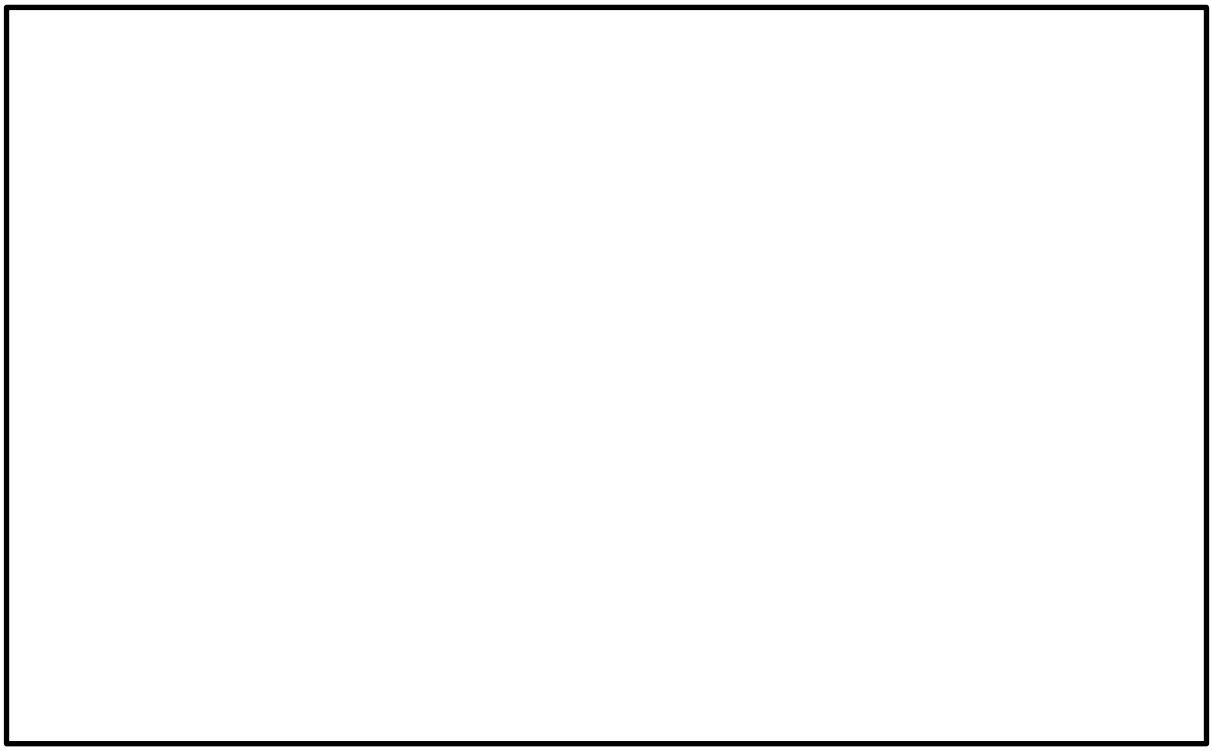

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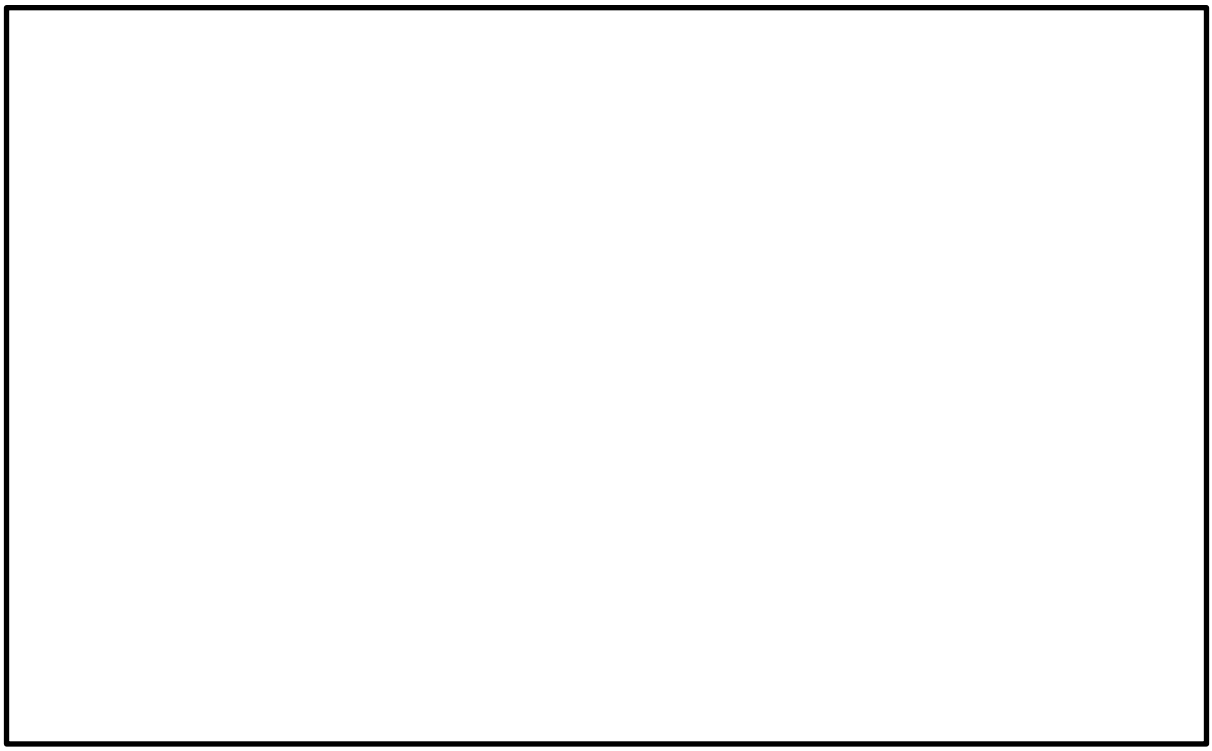

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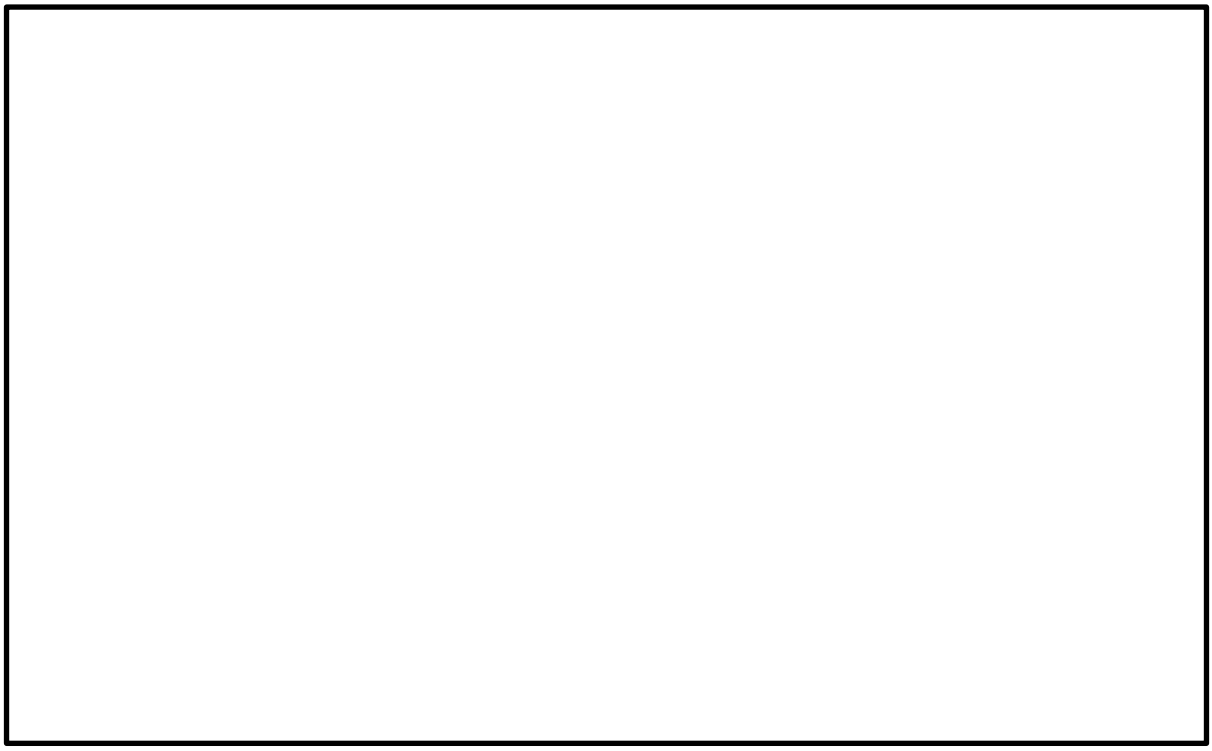
Practical No. 11

Objective: To study about floral biology of cross and often cross-pollinated commercial crop species.

Exercise: Dissect the flowers given to you (Cotton, Tobacco and Sugarcane) and sketch the diagram of inflorescence, single flower and separate floral parts.



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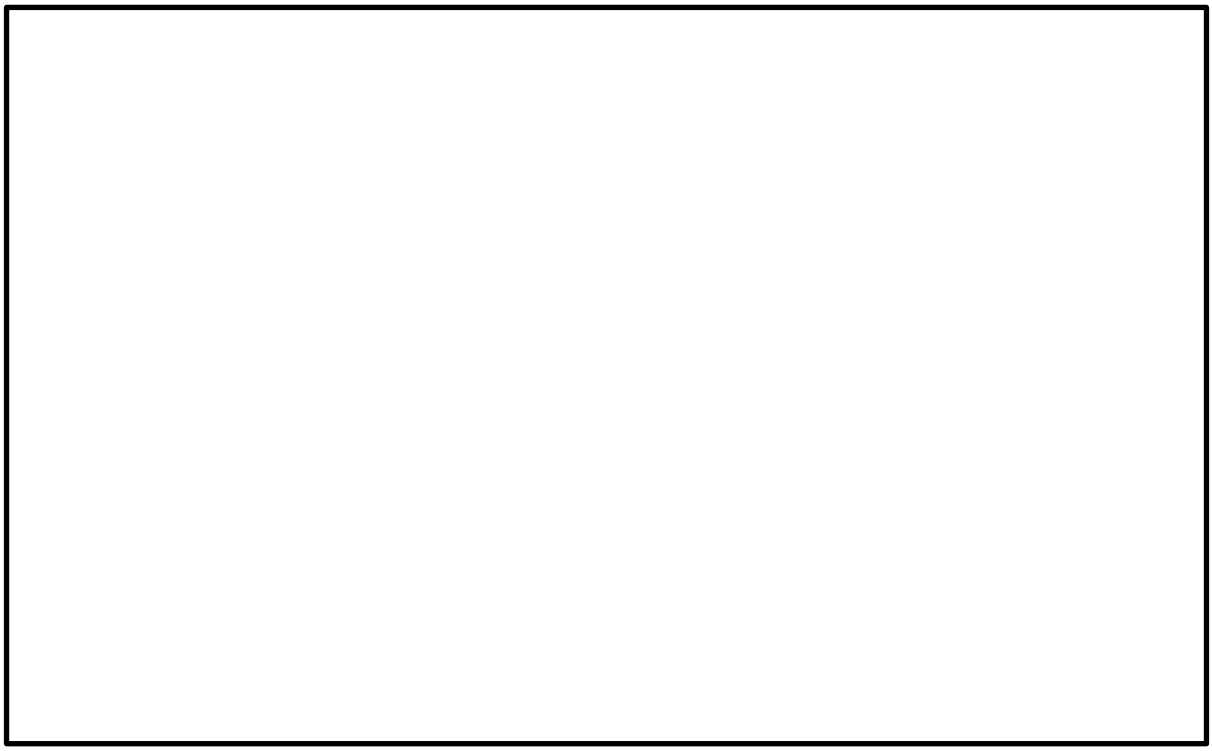

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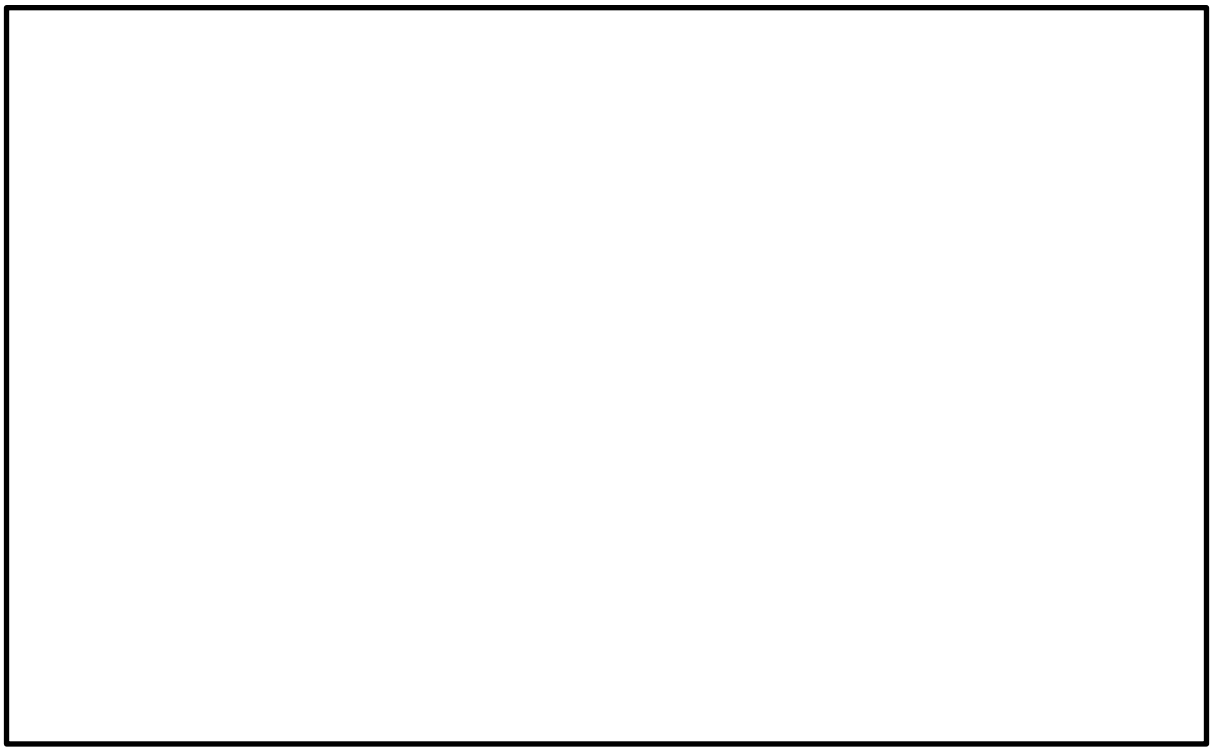

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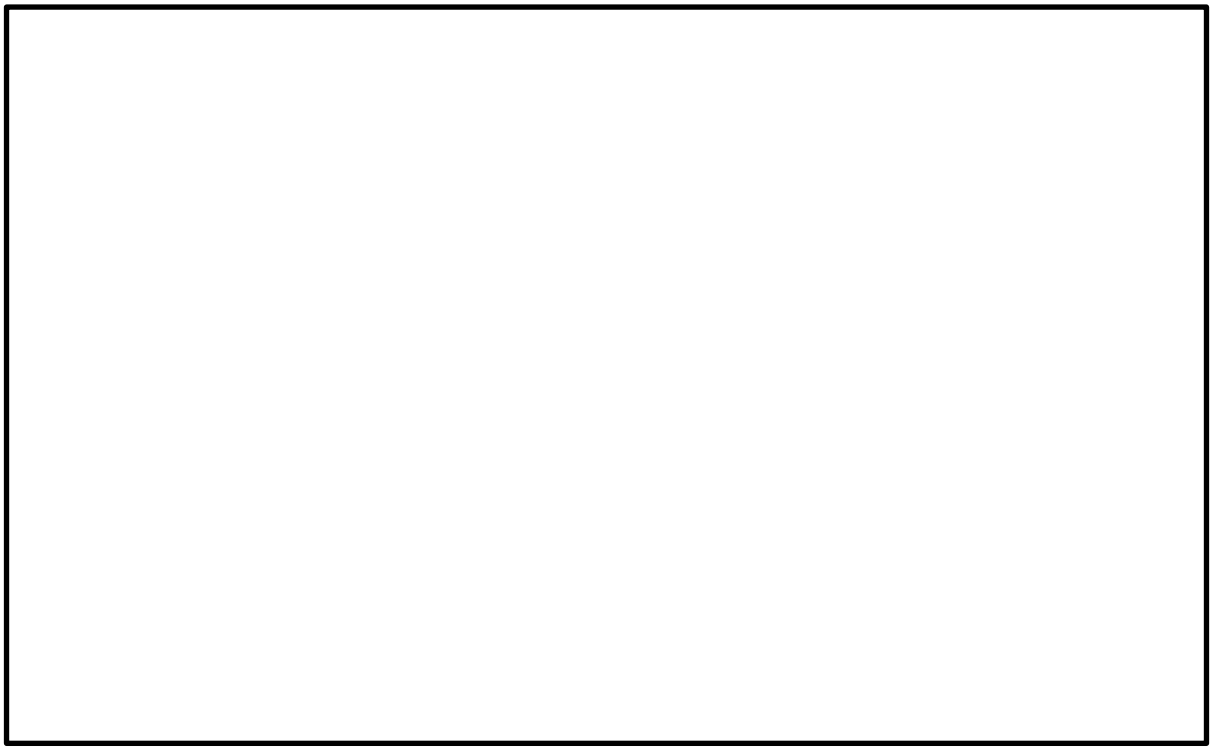
Practical No. 12

Objective: To study about floral biology of cross-pollinated Vegetable crop species.

Exercise: Dissect the flowers given to you (Onion) and sketch the diagram of inflorescence, single flower and separate floral parts.



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Practical No. 13

Objective: To study selfing and crossing techniques in crop plants and breeder's kit.

Exercise: Practice the selfing and crossing in field and draw the diagrams of various selfing, emasculation and crossing techniques used in different field crops.

Practical No. 14

Objective: To study Male sterility system in hybrid seed production of field crops.

Exercise: Diagrammatically represent hereditary constitution of the nucleus and cytoplasm of the different component lines of CMS, GMS, CGMS and Transgenic GMS (TrGMS) systems.

Practical No. 15

Objective: To study learning techniques in hybrid seed production using male sterility in field crops.

Exercise: Record the observations for pollen fertility count on A, B and R lines of Indian Mustard.

Component Lines	Number of Pollen Grains		Total	Percentage of Pollen fertility
	Unstained	Stained		
A- Line				
B- Line				
R- Line				

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Practical No. 16

Objective: To study optimization of hybrid seed production.

Exercise: Visit to hybrid seed production field and practice roguing and supplementary pollination.

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Practical No. 17

Objective: To study role of pollinators in hybrid seed production.

Exercise: Estimate the effects of pollinator insects in Indian mustard hybrid seed production field-

Treatment	No. of siliqua per plant	No. of seeds per siliqua	Yield Per plant (gm)	Seed yield per plot (Kg)
Open Pollination (Control)				
Cage with insect net				
Open pollination with Honey bee hive				
Increase over control (%)				

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Practical No. 18

Objective: To study purification of A/B/R lines and difficulties in hybrid seed production.

Exercise: Visit the field of nucleus seed production field of A, B and R lines and write the procedure of purification of A, B and R line.

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Practical No. 19

Objective: To study hybrid seed production in Rice.

Exercise: List down the popular hybrids of rice with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 20

Objective: To study hybrid seed production in Sorghum.

Exercise: List down the popular hybrids of sorghum with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 21

Objective: To study hybrid seed production in Pearl Millet.

Exercise: Enlist the popular hybrids of pearl millet with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 22

Objective: To study hybrid seed production in Maize.

Exercise: Enlist the popular hybrids of maize with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 23

Objective: To study hybrid seed production in Pigeon pea.

Exercise: Enlist the popular hybrids of pigeon pea with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 24

Objective: To study hybrid seed production in Sunflower.

Exercise: Enlist the popular hybrids of sunflower with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 25

Objective: To study hybrid seed production in Castor.

Exercise: Enlist the popular hybrids of castor with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 26

Objective: To study hybrid seed production in Cotton.

Exercise: Enlist the popular hybrids of cotton with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 27

Objective: To study hybrid seed production in Okra.

Exercise: Enlist the popular hybrids of okra with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 28

Objective: To study hybrid seed production in Tomato.

Exercise: Enlist the popular hybrids of Tomato with their pedigree, year and centre of release.

Popular Hybrids	Pedigree	Year	Centre of release

Practical No. 29

Objective: To study seed sampling and physical purity analysis.

Exercise: Prepare the various kind of sample from given seed lot.

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Practical No. 30

Objective: To study post-harvest handling of seed to maintain quality of seed.

Exercise: Visit the seed store godowns and learn the practical aspects of storage techniques.

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Practical No. 31

Objective: To study genetic purity testing.

Exercise: i) Learn the techniques of grow out test and draw the diagram of procedure.
ii) Calculate germination percentage of given samples.

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Practical No. 32

Objective: To study seed storage techniques.

Exercise: Learn the techniques of fumigation and seed treatment techniques in the seed godowns.

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Practical No. 33

Objective: Visit to the public seed production and processing units.

Exercise: Learn the seed production and processing techniques and submit the report of visit.

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Practical No. 34

Objective: Visit to the private seed production and processing units.

Exercise: Learn the seed production and processing techniques and submit the report of visit

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APPENDICES

FLORAL BIOLOGY OF SELF-POLLINATED CEREAL CROP SPECIES

Morphological and functional characteristics of flowers may have major effects on their reproductive success. The study of floral structure gives the knowledge of different flower parts (sepals, petals, stamens, pistil etc.) their number and position. The study of floral biology includes the behaviour of flowering, anther dehiscence, stigma receptivity and pollen viability. Floral biology of a crop species is variable and controlled by the factors like variety, nutrition and environmental factors (temperature and humidity). An understanding of flower structure and floral biology is necessary for a plant breeder to effectively control the pollination (either through selfing and crossing) and for the manipulation of the crossing procedure based on the mode of reproduction. The sequential process of flowering is called *'Anthesis'*. The knowledge of floral structure and floral biology of a crop is necessary to devise efficient breeding procedures. In order to carry out the crop improvement programme successfully. Therefore, we shall study the floral structure and the floral biology of some important self-pollinated crops:

CEREALS

WHEAT (*Triticum spp.*) (X = 7, 2n = 14, 28, 42)

Class: Monocotyledon, **Sub-Class:** Glumiflorae, **Series**: Glumacea, **Family:** Poaceae (Graminae), **Sub-family:** Pooideae, **Tribe:** Hordeae, **Genus:** *Triticum*, **Species:** *aestivum, dicoccum, durum*

Floral Structure: The inflorescence of wheat is called Ear/Head and in botanical terms is known as **'Spike'**. The spike consists of separate group of florets (flowers) known as a **'Spikelet'**. Spike of wheat is a determinate and composite spike bearing two rows of lateral sessile spikelets arranged singly in an alternate order at nodes of the central strong zig-zag axis (rachis). Two to five florets are borne in each spikelet of common wheat and florets are subtended by a pair of glumes. Each floret consists of a flowering glume (lemma) and a thin two keeled glume (palea). Each floret contains three anthers, an ovary (monocarpellary, superior ovary, unilocular) with **bifurcated** short styles and feathery stigma and two small lodicules (triangular). The glumes, lemma and palea are opened due to swelling of lodicules through absorption of moisture from atmosphere. This enables the florets to open and bloom.

Anthesis / Floral biology:

- Blooming starts flowering several days after the wheat spike emerges from the flag leaf.
- The florets on the main culm flowering first and those on the tillers flowering late.
- Blooming starts at approximately two third from the base and proceeds in both the directions.
- Flowering continues throughout the day, but the peak flowering takes place in the morning from 9:00 am to 1:00 pm. Two to three days required for a spike to finish blooming.
- The glumes, lemma and palea are opened due to swelling of lodicules through absorption of moisture from atmosphere. This enables the florets to open and bloom. The anthers protrude from the glumes; and part of the pollens is shed outside of the flowers.
- While the flower is open, foreign pollen may enter and resulting cross pollination normally up to extent of 1 to 2 %. If weather conditions are not favorable for the opening of glumes, the anthers may shed the pollen without being extruded.

PADDY / RICE (*Oryza sativa*) (2n = 24)

Class: Monocotyledon, **Sub-Class:** Glumiflorae, **Series:** Glumacea, **Family:** Poaceae (Graminae), **Sub-family:** Pooideae, **Tribe:** Oryzeae, **Genus:** *Oryza*, **Species:** *sativa*

Floral Structure: The inflorescence of rice is a terminal panicle that is composed of single flowered spikelets. The spikelets are borne on secondary branches. Spikelets vary from 50-500 in the panicle. Each spikelet consists of two sterile lemmas, the rachilla and the floret. The rachilla is small axis between the rudimentary glumes (the sterile lemmas) and the fertile floret. The floret includes the lemma, palea and the floret. The rice flowers consist six stamens (bilobed anthers) unlike three in other common cereal and gynoecium (monocarpellary, superior ovary, unilocular and plumose bifid stigma). At the base

of the ovary, two thin membranous and hygroscopic, triangular structures called as lodicules are present which helps in flower opening. Flower is surrounded by a lemma and palea, structures that form the hull or husk that enclose the grain (caryopsis).

Anthesis / Floral biology:

- The blooming of rice normally occurs between 10:00 am and 2:00 pm.
- The flowers in a single panicle take a period of 3 to 7 days to finish blooming, with most of the flowers bloom on second and third day after the emergence of the panicle from the flag leaf. The time and rate of blooming varies with the genotype and is affected by the environmental factors and (light, temperature and humidity).
- Blooming starts from the terminal spikelets and proceeds towards the lower ones (basipetal succession).
- Anthers dehisce and shedding of pollen generally takes at the time the flower opens with blooming of the spikelet.
- The stigma remains maximum receptive during the first three days after opening of the spikelet's, which gradually decreases and lost within seven days. While, rice pollen grains remain viable only for five minutes. Therefore, pollen grains germinate on stigma in about few minutes after the pollination.
- The rice flower is normally self-pollinated and the extent of natural crossing varies from zero to 3 % with an average of about 0.5 %, depending upon genotype and environment.

BARLEY (*Hordeum vulgare L.)* (2n= 2x= 14)

Class: Monocotyledon, **Sub-Class:** Glumiflorae, **Order:** Poales, **Family:** Poaceae (Graminae), **Sub-family:** Pooideae, **Tribe:** Triticeae, **Genus:** *Hordeum*, **Species:** *vulgare*

Floral Structure: The barley inflorescence is referred to as ear, head and botanically spike. The flowering units, spikelets are attached directly to the central axis, or rachis, which is the extension of main stem that supports the spike. There are three spikelets at each node, called triplets alternating on opposite sides of the spike. Each spikelet is single flowered and made up of two glumes, which are empty bracts, and one floret that includes the lemma and the palea, the enclosed reproductive components. Depending on cultivar, each lemma is extended as an awn, or more rarely a hood. In most of the varieties, the glumes are about one-half the length of the lemma and terminate in a slender awn. In six-rowed barley, all the spikelets in triplet are fertile and able to develop into grains and spikelets are attached at each node of the rachis, and these triplets alternate from side to side of the rachis. In two-rowed barley, only the central spikelet of a triplet is fertile and lateral spikelets being sterile or vestigial. The pistil has a two branched feathery stigma. Three anthers are attached to the long slender filaments. Lodicules present below the ovary helps in opening of the flower. In hulled or husked varieties, the palea and lemma adhere to the grain, in hull-less of naked varieties, the palea and lemma are not attached and separate from the grain on threshing.

Anthesis / Floral biology:

- Flowering begins in the mid florets of the upper part of the spike and proceeds both up and downwards of the spike.
- As anthesis approaches, the lodicules absorb the moisture, the flower opens and the filaments elongate.
- The anthers dehisce as they emerge from the flower, and pollen is spilled upon the stigma. Barley pollen germinates within five minutes of adhering to stigma and pollen viability estimates range from a few hours to at least one day, while stigmas are receptive for few days (3-5 days).
- The extent of natural outcrossing in barley varies from 0 to 9.6 %, with a low overall average of 1.5 %.

FLORAL BIOLOGY OF SELF-POLLINATED MILLET CROP SPECIES

FINGER MILLET / RAGI (*Eleusine coracana* G.) (2n = 36)

Class: Monocotyledon, **Sub-Class:** Glumiflorae, **Series:** Glumacea, **Family:** Poaceae (Graminae), **Sub-family:** Pooideae, **Tribe:** Chlorideae, **Genus:** *Eleusine*, **Species:** *coracana*

Floral structure: Finger millet inflorescence is borne on log peducncles, consisting in the whorl of 2-11 digitate, straight or slightly curved spikes called fingers. The spikelets are sessile, generally with 4-6 florets and subtended by two glumes. The flowers are perfect except for the terminal flower which may be either staminate or sterile. Each perfect flower contains lemma, palea, three stamens, an ovary (bi-carpellary, uni-locular, superior ovary) with feathery branched stigma and two scaly lodicules.

Floral Biology:

- Within a spike, spikelet opens from the top to down- ward (besipetal) while within a spikelet floret opens from bottom to top (acropetal) and one floret in a spikelet opens per day.
- The maximum number of flowers opens on the third day after initiation of flowering. It takes a week to finish blooming.
- The anthesis occurs between 1.00 to 5.00 a.m. As soon as lemma and palea begins to gap, the stigma and emerge concurrently to ensure 100 % self-pollination.
- The anthers dehisce and pollinate their own stigmas. Pollen grains remain viable for 20 minutes while receptivity of the stigma is up to 5 hours.

- Extent of natural crossing does not exceed 1 % in finger millet.

KODO MILLET (*Paspalum scrobiculatum* L.) (2n= 4x= 40)

Class: Monocotyledon, **Family:** Poaceae, **Genus:** *Paspalum*, **Species:** *scrobiculatum*

Floral Structure: Kodo millet inflorescence comprises of 2-6 racemes spreading widely on a sub-digitate or a short axis. The spikelets are usually sessile or on a short pedicel. They are usually single arranged in two rows on a flattened rachis. In the middle of the raceme, some spikelets are paired. The spikelets are arranged alternatively in two series i.e. long and short pedicelled. The glume I is absent and the glume II is equal to the length of spikelet. The lemma I is almost similar to glume II while lemma II encloses both the two florets. The lower floret in the spikelet is sterile and reduced to valve, while the upper one is a hermaphrodite flower. Stamens are three in number, filamentous with three bi-lobed anthers. Gynoecium is monocarpellary, ovary is superior, one cell with one ovule, two stigmas, feathery distinct style. The grain is enclosed in hard horny persistent husks.

Anthesis / Floral biology:

- Spikelets located on the middle of the raceme opens first and gradually spread to both ends.
- Spikelet opens between 2:00 a.m. to early morning and the flowering in a panicle takes nearly one week to complete.
- The stigma comes first during blooming of flowers and anthers arise just after the emergence of stigma.
- Kodo millet has a cleistogamous flower and the percentage of open flowers does not exceed 15-20% thereby, self-pollination is the rule. Protogyny also exists in some of the Kodo millets cultivars like 'IPS 147', 'IPS 197', 'IPS 427' etc.

BARNYARD MILLET (*Echinochloa frumentacea* L.) (2n=6x=54, x=9)

Class: Monocotyledon, **Family:** Poaceae, **Genus:** *Echinochloa*, **Species:** *frumentacea*

Common name- Sawa millet or billion-dollar grass

Floral Structure: Barnyard millet inflorescence is long terminal panicles that are usually erect with different shapes, viz. cylindrical, pyramidal and globose to elliptic. The panicle

consists of few to numerous, densely crowded racemes with spikelets arranged in 4 irregular rows on the triquetrous rachis. Spikelets can be awned or awnless, depending on the species. Pedicillate spikelets are enfolded by unequal glumes, the upper glume is as long as the spikelet and consists of two florets- lower sterile (with lemma and small palea), and the upper fertile bisexual floret which has thick broad lemma and palea, two lodicules, three stamens and superior ovary with two plumose stigmas.

Anthesis / Floral biology:

- The flower opens in the upper raceme first and flowering occurs from tip of the inflorescence towards the base.
- The panicle takes 10-14 days for emergence and takes 10-15 for completion of flowering and in case of compact panicles it requires 3-4 days more.
- Flowers open from 5-10 a.m. with maximum number of flowers opens between 6-7 a.m.
- In the individual spikes, the spikelets along the two margins open earlier than those at the middle raceme.
- The flowers open out gradually with the stigmatic branches spreading out followed by the anthers and then the dehiscence of the anthers. The flower closes within half an hour.
- Kodo millet is basically an autogamous species, but some percentage of cross pollination is also reported mainly via wind.

FLORAL BIOLOGY OF SELF-POLLINATED PULSE CROP SPECIES

BENGAL GRAM / CHICKPEA (*Cicer arietinum* L.) (2n = 16)

Class: Dicotyledon, **Sub-Class:** Polypetalae, **Series:** Calyciflorae, **Order:** Rosales, **Family:** Fabaceae (Leguminoceae), **Sub-family:** Papilionaceae, **Tribe:** Vicieae, **Genus:** *Cicer*, **Species:** *arietinum*

Floral structure: Flowers are papilionaceous, zygomorphic, solitary in axillary racemes and peduncle is jointed. The calyx has five deep lanceolate teeth. Calyx and peduncle are densely covered with hairs. Corolla (petals) is five (2+2+1) in number and 1cm long, greenish white to pink or blue in colour, standard petal is broad and clawed, wings free, keel incurved. Stamens are ten in number and diadelphous (9+1) in condition. Ovary is superior with glabrous style and globose type of stigma.

Anthesis / Floral biology:

- In gram or chickpea, anthesis starts between 9-10 am and may continue up to 3 pm.
- The flowers open in two successive days and the flowering process will be over early on the second day.
- The plant is primarily self-pollinated due to cleistogamy and anthers dehisce about 40 hours prior to opening of flowers.
- A very small percent of cross-pollination may result from insect visitation after the flowers open.

GREEN GRAM (*Phaseolus aureus* or *Vigna radiata* (L.) Wilczek.) (2n = 22)

Class: Dicotyledon, **Sub-Class:** Polypetalae, **Series:** Calyciflorae, **Order:** Rosales, **Family:** Fabaceae (Leguminoceae), **Sub-family:** Papilionaceae, **Genus:** *Vigna*, **Species:** *radiata*

Floral Structure: The inflorescence is terminal or axillary receme carrying more than 10 flowers with long pedicels on each peduncle. Flower is bracteate, bisexual, complete, regular, zygomorphic, hypogynous and pentamerous. The flower is a typical papilionaceous with 5 Sepals (three large and free and two small and fused), five petals (keel petals are spirally coiled) 10 diadelphous (9+1) stamens (filaments alternately long and short and anthers uniform), Gynoecium is monocarpellary, unilocular, superior ovary with many ovules and hairy style.

Floral biology: In green gram, flower opening occurs between 6-7 a.m. and the flowering continues for an hour or two. They remain fully open until noon and they gradually close, being completely closed by 2-4 pm.

- The pollination occurs a night prior to opening of the flowers and the anthers start dehiscing from 9 a.m. and completely dehisced by 3 a.m.
- The petals will shed in the following morning.
- In green gram self-pollination is the rule and cleistogamy is prevalent to a greater extent.

BLACK GRAM (*Phaseolus mungo or Vigna mungo)* (2n=2x=22)

Class: Dicotyledon, **Sub-Class:** Polypetalae, **Series:** Calyciflorae, **Order:** Rosales, **Family:** Fabaceae (Leguminoceae), **Sub-family:** Papilionaceae, **Genus:** *Vigna*, **Species:** *mungo*

Flower Structure: Inflorescence is axillary raceme, may have 2-3 branches and 5-6 flowers clustered at the top of hairy peduncle. Bracteoles are longer than calyx. Flowers are typical papilionaceous with five sepals (gamosepalous, 5 sepals united), 5 petals (2+2+1, keel petal in the form of beak), 10 diadelphous stamens (9+1) and Gynoecium is monocarpellary, unilocular, superior ovary, unilocular with five ovules on marginal placentation, style is spirally twisted.

Floral biology:

- The flower opens early in the morning (6:00 am to 7:00 am) and the flowering continues for an hour or two.
- The anthers begin to shed pollen in the evening and it is completed by midnight in the closed bud and flowers open after pollination as receptivity of stigma is also attained at the time of anthesis.
- In black gram self-pollination is the rule and cleistogamy is prevalent to a greater extent.

FLORAL BIOLOGY OF SELF-POLLINATED PULSE CROP SPECIES

FIELD PEA (*Pisum sativum* L.) (2n=2x= 14)

Class: Dicotyledon, **Sub-Class:** Polypetalae, **Series:** Calyciflorae, **Order:** Rosales, **Family:** Fabaceae (Leguminoceae), **Sub-family:** Papilionaceae, **Genus:** *Pisum*, **Species:** *sativum*

Floral Structure: The inflorescence is axillary raceme arising from axil of a leaf. Flowers are typical papilionaceous with green calyx comprising of five united sepals, 5 petals (2+2+1), 10 diadelphous stamens (9+1, nine filaments are fused to form a stamina tube while tenth is free throughout its length) and Gynoecium is monocarpellary, with ovules (up to 13) alternately attached to the two placentas. Style normally bends at right angle to the ovary. Stigma is elliptical and sticky.

Anthesis / Floral biology:

- The flowers open early in the morning (6:00 am to 7:00 am) and continue till 11:00 am.
- The anthers dehiscence takes place just before flower opening and stigma is covered with full of pollen grains at the time of flower opening.
- Pollen viability – 5 to 6 hours and stigma receptivity for 1-2 days.
- It is predominantly self-pollinated crop because of chasmogamous nature and only 0.5 to 3 % cross pollination can occur due to insects.

LENTIL (*Lens culinaris* ssp. *Culinaris* Medik.,) (2n= 2x= 14)

Class: Dicotyledon, **Sub-Class:** Polypetalae, **Series:** Calyciflorae, **Order:** Rosales, **Family:** Fabaceae (Leguminoceae), **Sub-family:** Papilionaceae, **Genus:** *Lens*, **Species:** *culinaris*

Floral Structure: Flowers are borne single/double, sometimes in threes and rarely in four in axillary raceme with short slender peduncles. The flower is typical papilionaceous, small, white, pale purple or purple blue and flowering proceeds acropetally. Flowers are typical papilionaceous with green calyx (5, companulate, 5 lobed, narrow, sub equal), 5 petals (2+2+1), 10 diadelphous stamens (9+1, nine filaments are fused to form a stamina tube while tenth is free throughout its length and anthers are basifixed) and ovary is flat and non-pubescent usually with two ovules.

Anthesis / Floral biology:

- The flowers open early in the morning (6:00 am to 7:00 am) and continue till 11:00 am.
- The anthers dehiscence takes place just before flower opening and stigma is covered with full of pollen grains at the time of flower opening.
- It is predominantly self-pollinated crop because of chasmogamous nature.

FLORAL BIOLOGY OF SELF-POLLINATED OILSEED CROP SPECIES

SOYBEAN (*Glycine max (*L.) Merr.) (2n = 4x= 40)

Class: Dicotyledon, **Sub-Class:** Polypetalae, **Series:** Calyciflorae, **Order:** Fabales,

Family: Fabaceae (Leguminoceae), **Sub-family:** Papilionaceae, **Genus:** *Glycine*, **Species:** *max*

Floral structure: Inflorescence bears flowers in clusters of 3-15 flowers in the axil of a branch. Many flowers are shed without forming pods. The flowers are characteristic of the legume family, the calyx is tubular with 5 unequal sepals, corolla consisting of five petals (2+2+1), which enclose a pistil and 10 diadelphous stamens. The pistil is unicarpellary and has a single ovary with generally two or four ovules, a long style curves backward towards the free posterior free stamen. Hairs are present on the pistil, and the outer surface of the calyx tube.

Anthesis / Floral biology:

- The flowers open normally early in the morning, but opening may be delayed in cool weather.
- Pollen from the anthers is shed directly on the stigma before opening of the flower resulting in a high degree of self-pollination and less than 1 % of natural cross pollination occurs**.**

SESAME/TIL (*Sesamum indicum*) (2n= 26)

Class: Magnoliopsida (Dicotyledons), **Sub-Class:** Asteridae, **Order:** Lemiales,

Family: Pedaliaceae **Sub-family:** Papilionaceae, **Genus:** *Sesamum*, **Species:** *indicum*

Floral Structure: The flowers are borne in clusters on small peduncles in axils of leaves. Flowers are solitary or in a group of two or three. The calyx is gamosepalous of five lobes and usually green in colour but in some cultivars it is red brown to slightly yellow. Corolla is tubular with an entire upper lip and three lobed lower lip. The middle is larger than the other two side lobes and coloring of various shades of purple white. Flowers are variously colored. The stamens are four in number, epipetalous, didynamous (two large and two short), anther sacs are dorsi-fixed and dehisce longitudinally. Gynoecium has superior ovary, bicarpellate or tetracarpellate or many ovules.

Anthesis / Floral biology:

- The flower opening is in acropetal succession. The flowers open in early morning and period lasts from 5 a.m. to 7 a.m.
- The anthers start dehiscing at 3:00 am to 4:00 a.m. shortly before the flower opens. Pollen viability is for few hours.
- The stigma is receptive at the same time as the dehiscence of anthers and remains receptive for two more days if not fertilized.
- Fruit is capsule and each capsule contains 50 to 100 or more seeds depending on cultivar.

GROUNDNUT (*Arachis hypogeae* L.) (2n = 40)

Class: Dicotyledon, **Sub-Class:** Polypetalae, **Series:** Calyciflorae, **Order:** Rosales, **Family:** Fabaceae (Leguminoceae), **Sub-family:** Papilionaceae, **Genus:** *Arachis,* **Species:** *hypogeae*

Floral structure: Flowers occupy either solitary or in clusters of about three or more from the leaf axils. In case of bunch type of groundnut, large number of flowers arises at the basal branches. In spreading types, the flowers are found on the procumbent branches throughout. Flowers are sessile, yellow in colour and carried on the fairly long calyx tube which gives false appearance of a pedicel; single bract and two bracteoles are present for each flower. Calyx is tubular, slender, ending in five lobes of which 3 are united into one big structure and the other two are linear lanceolate. Corolla is papilionaceous arising on the rim of the calyx tube, keels are united. Stamens are monodelphous (10) of which eight fertile and two sterile, staminodes and 8 fertile, stamens are dimorphic (4 long anther lobed and 4 round). Gynoecium with superior ovary, the style is very slender and passes through the length of the calyx tube and the monodelphous staminal bundle, style is long and filiform and has hairs on its distal portion. Stigma is terminal and hairy. Ovary is monocarpellary, unilocular with 1-3 ovules; marginal placenta. Fruit is indehiscent pod (wrongly called a nut) carried on a long stalk which is the gynophore (more correctly **carpophore**). After fertilization, an intercalary meristem at the base of ovary becomes activated and the carpophore begins to grow with the ovary at its tip and known as Peg.

Anthesis / Floral biology:

- Flowering generally begins about 25 days after sowing. In spreading types, flowering is delayed by about 7 days. Flowering is observed for about 30 days.

- The dehiscence of anthers takes place between 3-5 am and flowers open between 6-8 am (two hours prior to flower opening ensuring self-pollination).
- Receptivity of stigma is up to 3-4 hours after flower opening.

FLORAL BIOLOGY OF SELF-POLLINATED OILSEED CROP SPECIES

LINSEED/ Flax (*Linum usitatissium*) (2n= 2x= 30)

Class: Magnoliopsida (dicotyledons), **Sub-Class:** Rosidae, **Order:** Linales,

Family: Linaceae **Genus:** *Linum* **Species:** *usitatissium*

Floral Structure: The inflorescence is simple cymose and recemose type, flowers are perfect, actinomorphic, pentamerous, 5 sepals and petals are free. The androecium has ten Stamens present in two whorls, but the outer whorls consists five stamens being reduced to staminodes. The inner five fertile stamens are widened to form a fused ring which surrounds the base of the gynoecium. The superior gynoecium consists five united carpels, ten loculed due to false septum in each carpel, each locule with single ovule. Ovary has axile placentation and ovules are pendulous and anatropus with five styles (filiform) arising from the ovary twisted together and with the terminate capitate stigma.

Anthesis / Floral biology:

- Blooming starts at 6 a.m. and is completed by 8 a.m.
- The pollen is viable for only a few hours, from the time of anther dehiscence until about the time the petals dehisce - between 4 and 7 hours
- Dehiscence starts at 7 am and stigmatic surface is completely covered with pollens by 10 am.
- As the flower opens, the anthers come together and form a cap over the stigma. Stigma remains receptive for a period of 2 hours before and 5 to 6 hours after opening of the flower.
- Petals with and fall apart by 11 am.
- Linseed predominantly self-pollinating but cross pollination may occur up to the 2 % by the insects.

RAPESEED & MUSTARD (*Brassica spp.*) (2n = 16-38)

Class: Magnoliopsida (dicotyledons), **Order:** Brassicales, **Family:** Brassicaceae/ Cruciferae, **Genus:** *Brassica*, **Species:** *campestris, napus, juncea, rapa, nigra, carinata*

Floral structure: The flower has typical cruciferae formula [(K2 +2, C4, A2+4, G(2)]. The inflorescence is a recemose and flowering is indeterminate beginning at the lowest bud on the main raceme. Flowers are small, ovary hypogynous, bicarpellary (separated by a false septum), syncarpous with a very large number of ovules on parietal placentation. The fruit is siliqua.

Anthesis / Floral biology:

- Blooming starts from 8 am and continues till noon. Flowers remain open for 2 to 3 days and on the third and fourth day, the sepals, petals, and stamens are dropped.
- The stigmas become receptive even two to three days before the flower opens.
- Pollen grains in anthers are viable for 24 to 48 hours.
- The amphidiploid species (*Brassica juncea, B. napus* and *B. campestris* var. yellow sarson and *B. carinata*) are self-compatible and self-pollinated. However, up to 30 % natural cross pollination may through the insects (bees) and wind. The diploid species (*B. nigra, B. oleracea* and *B. campestris* var. toria) are self-incompatible (sporophytic and homomorphic) and consequently cross pollinated.

FLORAL BIOLOGY OF SELF-POLLINATED VEGETABLE CROP SPECIES

TOMATO (*Lycopersicon esculentum* Mill.) (2n = 2x= 24)

Class: Magnoliopsida (dicotyledons), **Order:** Brassicales, **Family:** Solanacae, **Genus:** *Lycopersicon*, **Species:** *esculentum*

Floral Structure: Inflorescence is an extra-axillary cyme with dichotomous or polychotomous branching, there are 4-8 flowers in each compound inflorescence. There is light protective anther cone surrounding stigma leading to self-pollination. The first flower is always bigger than the others. The flowers are bisexual, actinomorphic, small, hypogynous and yellow in colour. Calyx comprises of 5 sepals which are core united and often persistent and possesses trichomes, corolla consists of 5 petals and gamopetalous, stamens (6) are epipetalous with small filament and large anthers. These form a cone and enclose the pistil. Ovary is superior, bicarpellary and syncarpous, single style with bilobed stigma.

Anthesis / Floral biology:

- Flowering occurs early in the morning, around 6 am to 11 am and maximum flower opening occurs between 7 am to 9 am.
- Anther dehiscence also concurrently occur with flower opening.

- Stigma is receptive at the time of anthesis as stigma becomes receptive 16 to 1 hours before anthesis to 5 to 6 days after anthesis. Pollens remain viable for 2 to 5 days (1-25 ^{0}C).
- Essentially a self-pollinated crop as stamens form a solid cone enclosing the pistil but when the style protrudes above the anther tube, chances of cross pollination through bees increases.

OKRA/BHINDI/LADIES FINGER (*Abelmoschus esculentus*)

Class: Magnoliopsida (Dicotyledons), **Order:** Malvales, **Family:** Malvaceae, **Genus:** *Lycopersicon*, **Species:** *esculentus*

Floral Structure: Flowers are solitary, axillary with long peduncle and having epicalyx up to 10. There are five yellow petals with crimson spot at the base of each petal and flowers wither within one day. Staminal column consists of numerous stamens which are united to base of petals. Stigma is 5-9 lobed and fruit is capsule.

Anthesis / Floral biology:

- Flowering starts from bottom to upwards. Anthers dehisce before flower opening and hence self-pollination may occur at anthesis.
- The dehiscence of anthers is transverse and complete dehiscence occurs in 5-10 minutes.
- Pollen fertility is maximum in the period between an hour before and an hour after opening of flower.
- Flowers remain open for shorter duration and wither in afternoon. The stigma is receptive during anthesis; hence pollination is not very successful at bud stage. The okra is self-pollinated crop by rule but cross pollination up to the extent of 4-19 % with maximum of 40 %.

CHILLI (*Capsicum annuum*) and Bell pepper (*Capsicum frutescens*) (2n=2x=24)

Class: Magnoliopsida (Dicotyledons), **Order:** Solanales, **Family:** Solanaceae, **Genus:** *Capsicum* **Species:** *annum*

Floral structure: Flowers are solitary and axillary tending to be opposite to leaf. Sometimes, flowers occur in pairs, flower is bracteolate, pedicillate, bisexual, zygomorphic. Calyx is campanulate, sepals 5 (gamosepalous), corolla usually white, petals 5 and gamopetalous. Androecium is with 5 stamens, epipetalous. Ovary is superior, bicarpellary, syncarpous, bicolour with number of ovules, single style, and stigma sub-capitate and faintly bifid.

Anthesis / Floral biology:

- The average life of a flower after the opening is 2-3 days, then attaches and become fruit or dries up and falls off.
- The time of flower opening is 11 am to 1 pm and varies with the environmental conditions. Majority of the buds open between 8-10 am.
- Anther dehiscence takes place about half an hour after the opening of the flowers.

FLORAL BIOLOGY OF SELF-POLLINATED VEGETABLE CROP SPECIES

POTATO (*Solanum tuberosum* L.) (2n = 4x= 48, x= 12)

Class: Magnoliopsida (Dicotyledons), **Order:** Solanales, **Family:** Solanaceae, **Genus:** *Solanum* **Species:** *tuberosum*

Floral Structure: The inflorescence is monochasial cyme with few flowers and arises from extra axillary position. Flowers are actinomorphic, hermophrodite, pedicillate, bracteate, calyx gamosepalous, 5 lobed, corolla gamosepalous, tubular and five lobed, rotary. Androecium consists of five stamens, epipetalous and anther dehiscence apically. Ovary is superior, bicarpellary, axil placentation, ovules many, style single, erect, capitate stigma.

Anthesis / Floral biology:

- Flowers in cultivated potato mostly open in the early morning, although a few may continue to open throughout the day.
- Pollen viability is for long periods may be desiccated and stored at low temperature up to one year.
- Cool, wet weather favours flower formation, while dry weather depresses flowering.
- Pollination takes place either by direct contact of anthers with stigma or through wind and insects.
- Most of the commercial potato varieties are pollen sterile, but the diploid species have abundant pollen.

BRINJAL/EGG PLANT (*Solanum melongena L.*)

Class: Magnoliopsida (Dicotyledons), **Sub Class-** Asteridae **Order:** Solanales, **Family:** Solanaceae, **Genus:** *Solanum* **Species:** *melongena*

Flower Structure: Brinjal flowers are large, violet coloured and solitary or in clusters of two or more. Flower consists of calyx: sepals 5, united, persistent; corolla: petals 5, united, usually cup shaped; Androecium is having five stamens, alternate with corolla; Gynoecium: carpels are united, ovary superior. The hypogynus gynoecium is syncarp located

obliquely in relation to the median. In most varieties the perfect flowers are borne singly and opposite the leaves. In brinjal heterostyly is a common feature.

Floral Biology:

- Usually, anthesis starts from 6 to 7.30 AM and continues up to 11 AM and peak time for anthesis is 8.30 to 10.30 AM. The pollen dehiscence starts from 9.30 to 10 AM.
- Stigma receptivity is highest during anthesis. The receptivity of the stigma could be observed from the plump and sticky appearance which gradually turns brown with the loss of receptivity.
- The stamens dehisce at the same time stigma is receptive so that self-pollination is a rule, although there is some cross pollination by insects also.
- The cone-like formation of anthers favors self-pollination; but since the stigma ultimately protrudes beyond the anthers, there is an ample opportunity for cross-pollination. The extent of cross-pollination has been reported as

FLORAL BIOLOGY OF CROSS-POLLINATED AND OFTEN CROSS-POLLINATED CEREAL AND MILLET CROPS

MAIZE (*Zea mays* L.) (2n = 20)

Class: Monocotyledon, **Sub-Class:** Commelinidae, **Order-** Cyperales, **Family:** Poaceae (Graminae), **Sub-family:** Pooideae, **Tribe:** Maydee, **Genus:** *Zea*, **Species:** *mays*

Floral structure: The corn plant is monoecious (both staminate and pistillate inflorescence) and diclinous (male and female inflorescence borne separately on the same plant). The male inflorescence is known as 'Tassel' occurs on terminal position of main axis and female inflorescence is botanically Spadix type and borne on a shoot located midway of the stalk and commonly known as 'Cob/Ear'.

The cob / ear is considered as a modified lateral branch originating from an axillary bud on the main stem. It is enclosed by husk and is made up of a thickened central rachis on which spikelets are borne in pairs, each spikelet normally containing one fertile and one sterile ovule, resulting in an even number of rows of kernals on the ear. Fertilization of the second ovule produces crowded and irregular rows of kernals on the ear. Pistil comprises three carpels, two extending to from the silk and third forming the ovule. The ovary ends in a thread like structure i.e., silk, which is actually a modified style and function as both stigma and style. The style is long and slender ranging from 35-40 cm in length and is receptive to fresh pollen throughout their entire length.

The main axis of maize plant terminates in a tassel and it is much-branched panicle, in which spikelets are arranged on both central axis of the panicle and on the branches in pairs, one sessile and other pediceled, both being identical in size, shape and structure. In each spikelet, there are two florets and each floret has a lemma, a palea, 3 stamens, 2 lodicules and a rudimentary pistil. Anthers are bilobed and protoandrous in nature due to this phenomena maize is cross pollinated by rule.

Anthesis / floral biology:

- Spikelets of central axis of tassel are blooming first and spikelets on lateral branches are blooming after 1 or 2 days.
- Blooming period varies from 2-14 days in different varieties.
- As the tassel opens, the anthers are pushed out by elongating filaments and pollen grains are emptied from the extruded anthers.
- Pollen shedding begins 1-3 days before the silks emerge from the shoots of the same and continues up to 3-4 days after the silk becomes receptive to pollen.
- A single tassel from a normal plant may produce 25,000,000 pollen grains, or an average of over 25,000 pollen grains for each kernel on an ear of corn.
- Fertilization occurs between 12-24 hours after the silk have been pollinated.
- Flower opening starts in the early morning and completes by afternoon.
- Pollen remains viable for about a day.
- Emergence of silks or styles takes 2-5 days and stigma receptivity is up to 14 days.

PEARL MILLET/ BAJRA (*Pennisetum glaucum* (L.) R. Br.) (2n=14)

Class: Monocotyledon, **Sub-Class:** Glumiflorae, **Series:** Glumacea, **Family:** Poaceae (Graminae), **Sub-family:** Panicoideae, **Tribe:** Paniceae, **Genus:** *Panicum*, **Species:** *glaucum*

Floral structure: Inflorescence is 15-60 cm long, dense, cylindrical and terminal compound spike. Spike consists of a strong solid central axis called rachis on which infinite numbers of fascicles are packed. Each fascicle consists of 2 to 5 spikelets and a whorl of bristles (30-40). Usually, only two spikelets of a fascicle are well developed and fertile. Spikelets are sessile or shortly pedicelled (ovate or oblong in form) and consists of two empty glumes which partially enclose the florets. A spikelet contains two florets, the lower floret is staminate and upper one is hermophrodite. Staminate floret is covered by hairy pointed lemma and carries three stamens with filiform filaments and versatile anthers, which dehisce longitudinally.

Hermophrodite floret has an ovate, broad lemma with acute apex, a small membranous palea, three stamens and one carpel containing superior ovary with single ovule. The style of ovary divides into two branches in its upper part and possesses stigmatic hairs. Lodicules are present in this floret also.

Anthesis / Floral biology:

- The flowering is protogynous in nature, where styles mature much before the anther to ensure high extent of cross-pollination.
- The ear heads start emerging after 30-40 days after sowing. The styles begin to protrude to open out the stigmas from top to bottom after 2-3 days of appearance of heads.
- Emergence of stigmas starts from top to bottom (basipetal).
- Maximum emergence of styles is observed between 9 am to 3 pm and styles remain receptive for 1 to 2 days.
- Before the basal stigmas protrude, stamens of hermophrodite flowers start emerging from tip to downwards.
- Before the emergence of stamens of bisexual florets reach the base of the earhead, the second flush of emergence of anthers from staminate flowers start from tip to downwards.
- Maximum anthesis is observed from 10 p.m. to 2 a.m., but pollen shedding continues throughout the day.
- Complete flowering of a spike commences in two phases i.e., anthers dehisce for 4-10 days in first round and about 20 days in the second.

SORGHUM (*Sorghum bicolor* L. Moench) (2n = 20)

Class: Monocotyledon, **Sub-Class:** Glumiflorae, **Series:** Glumacea, **Family:** Poaceae, **Sub-family:** Panicoideae, **Tribe:** Andropogonae, **Genus:** *Sorghum*, **Species:** *bicolor*

Floral Structure: The inflorescence is panicle / ear head. Peduncle consists of nodes and internodes on the central axis. Central rachis consists of many primary spikes, secondary spikes and tertiary spikes. In a spike, spikelets are in clusters of two (pairs) or three's, *i.e.*, one sessile floret (hermophrodite) and one or two pedicillate, which are rudimentary. Sessile floret consists of two glumes, one hairy lemma and non-haired lemma, small palea, three stamens, two lodicules and one pistil. Ovary is superior and has two styled feathery stigma.

Anthesis/Floral Biology:

- After 6 days of boot leaf initiation, panicle takes 8-9 days for emergence. Panicle / head needs 5 more days for its elongation.
- Complete flowering of panicle takes 7-8 days. But, maximum on 3rd or 4th day.
- Blooming starts in the uppermost spikelets and follows downwards (basipetal).
- Blooming starts during night or early morning (2 to 11 am).
- Anthers dehisce as soon as they come out and the pollen grains loose viability within few hours.

FLORAL BIOLOGY OF CROSS-POLLINATED AND OFTEN CROSS-POLLINATED PULSES AND OILSEED CROPS

PULSES

RED GRAM / PIGEONPEA (*Cajanus cajan*) (2n = 22)

Class: Dicotyledon, **Sub-Class:** Polypetalae, **Series:** Calyciflorae, **Order:** Rosales, **Family:** Fabaceae (Leguminoceae), **Sub-family:** Papilionaceae, **Tribe:** Phaseolae, Genus: *Cajanus*, **Species:** *cajan*

Floral Structure: Inflorescence is axillary or terminal racemes of umbels with two flowers at each node, butterfly shaped (vexillary aestivation) typically papilionaceous type. The outermost petal is too large and overlaps all other petals. The two laterals are called wings which overlap the two inner jointed petals, the keel. Keel encloses the stamens and gynoecium. Stamens are diadelphous (9+1), ovary pubescent, style incurved, stigma terminal and hairy.

Anthesis/Floral Biology:

- The flowers of pigeonpea normally open during the early morning and remain open for about 36-48 hours.
- Fertilization frequently occurs prior to complete opening of the flowers.
- In Hawaii, <1% natural cross pollination was reported but in India up to 25% has been recorded. So, by rule it is self-pollinated crops but it is coming under often cross-pollinated crop.

OILSEEDS

SUNFLOWER/ SURYAMUKHI (*Helianthus annuus* L.) (2n = 34)

Class: Magnoliopsida, **Sub-Class:** Asteridae, **Order:** Asterales, **Family:** Asteraceae**, Sub family**: Helianthoideae **Tribe:** Heliantheae, **Genus:** *Helianthus*, **Species:** *annus*

Floral structure: Inflorescence is head or capitulum with ray and disc florets. It is composed of many individual flowers in a large disc subtended by large flowers.

Disc florets are perfect flowers. These are bisexual, zygomorphic, tubular at the center, bracteoles at the base, calyx modified into scales (2). Corolla with five petals, gamopetalous and tubular. Androecium with 5 stamens, epipetalous and syngenecious. Gynoecium with two carpels and syncarpus. Ovary inferior with anatropous ovule, style is one and fruit is Cypsela.

Ray flowers are outer flowers, usually yellow and normally asexual but some may produce pollen. These flowers open first and flowering proceeds from periphery to the centre of the head.

Anthesis/Floral biology:

- It is protoandrous in nature where male parts mature first.
- Flowering starts from early morning 6-10am.
- Pollen viability is for an hour and stigma receptivity is for one day.
- Flowering starts at periphery and continues towards centre (centripetal) and is completed within 2-4 weeks.

SAFFLOWER *(Carthemus tinctorius*) (2n = 24)

Class: Magnoliopsida, **Sub-Class:** Asteridae, **Order:** Asterales, **Family:** Asteraceae, **Genus:** *Carthemus*, **Species:** *tinctorius*

Floral structure: Inflorescence is called as capitullum or head, terminal with involucres of bracts, oblong-acute. Florets are tubular, colour light yellow to orange yellow to red, bisexual, bracts and bracteoles of each flower modified into slender hairs, few florets at the periphery are sterile. Calyx is absent. Corolla is long, tubular divided at tip into 5 lobes, gamopetalous arising on the ovary. Stamens five, epipetalous, syngenecious (anthers united in a bundle or tube, but filaments are free). Ovary is inferior, bicarpellary, single celled and single ovuled.

Anthesis/Floral biology:

- On main branch flower blooms first and the last tertiary head flower in the order of their formation.
- It takes about 3-4 weeks for complete flowering in a plant.
- Within capitulum blooming begins in the outer circle of florets and progresses centripetally and the whole head completes flowering in about 3-4 days.
- The flowers open during the morning hours between 8-10 am.
- Since the stigma passes through the anther column usually it is completely covered with its own pollen. Therefore, it is self-pollinated crop predominantly. However, the insect population (generally bees) causes cross pollination of about 5-30 percent. So, it is also considered as often cross-pollinated crops.
- This is not wind pollinated since the pollen grains are sticky and aggregated.

FLORAL BIOLOGY OF CROSS-POLLINATED AND OFTEN CROSS-POLLINATED COMMERCIAL CROP SPECIES

COTTON (*Gossypium spp.*) (2n =2x= 26, 4x= 52)

Class: Magnoliopsida, **Sub-Class:** Dilleniidae, **Order:** Malvales, **Family:** Malvaceae**, Sub family**: Malvoideae **Tribe:** Gossypieae, **Genus:** *Gossypium*, **Species:** *arboreum, herbaceum barbadense and hirsutum*

Floral Structure: Flowers are extra axillary, terminal, solitary, opening of flower follows a spiral course in acropetal and centrifugal succession, cream, yellow or purple, borne on sympodial branches, rather large and showy. Bracteoles are three usually foliar and persistent, sometimes small or minute, cordate, toothed or entire. Bracts are free in American cotton and united in Asiatic cotton. Calyx is cup-shaped, truncate, undulate or shortly five-toothed, persistent. Corolla is tubular, five petals, white, creamy, yellow or purple. In some varieties, a spot of purple sometimes called 'eye' is found on the claw of the petals, not persistent. **Androecium**: Staminal column round the pistil bearing infinite number of filaments, the lower parts of filaments united into a tube, the upper free, unilocular and uniform anthers. **Gynoecium**: Ovary with 3–5 locules, often three, superior, ripening to dry, ovules one to several in each cell, often 20-30. Styles are glandular, club shaped or cleavate shortly into as many lobes as loculi in the ovary. **Fruit**: a loculicidal dehiscent capsule termed 'boll' with 3-5 locules. Seeds covered with one or two kinds of long unicellular hairs, in some wild species almost naked. 1-9 in each locule. The firmly adhearing hairs known as 'fuzz' and long detachable 'fibres' as lint. Lint and fuzz are outgrowths of epidermal cells on seeds. Lint is white, brown or green. Seed contains oil, protein and gossypol.

Anthesis/Floral Biology:

- Flowering begins about 40-45 days after sowing since the lowest branch is the oldest; the lower bud on each branch is the first to flower and the upper most the last. Anthesis is acropetal and centrifugal.
- The flower opens between 8 am to 12 noon.

- Dehiscence of anthers occurs between 8-11 am. Receptivity of stigma is for about 24 hours. The flower is generally pollinated shortly after it opens.
- The pollen grains germinate immediately, the tubes growing rapidly downwards through the conducting tissue of the base of the style. Fertilization is completed about 30 hours after pollination.
- Predominantly it is self-pollinated crop but amount of natural cross pollination by bees ranges between 5 to 50 percent therefore, it is often allogamous species.

TOBACCO (*Nicotiana tabacum* L.) (2n =4x=48)

Class: Magnoliopsida, **Sub Class:** asterids **Order:** Solanales, **Family:** Solanaceae**, Sub Family**: Nicotianodeae **Tribe:** Nicotianeae, **Genus:** *Nicotiana*, **Species:** *tabacum*

Floral structure: Inflorescence is simple raceme bearing as many as 150 flowers. Flowers are pedicillate and hermophrodite. Bisexual flowers bear 5 sepals forming calyx, corolla tube 10-15mm long and 2mm wide. Stamens inserted on the base of the corolla throat (epipetalous). Ovary is superior, bicarpellary, axil placentation, ovules many, style slender and capitate stigma.

Anthesis/Floral biology:

- Anther dehiscence completes by 8:00 am in *N. rustica* and by 10 a.m. to 12 noon in *N. tabacum* on the day of bud opening.
- Pollen remains viable for 24 hours and the stigma becomes receptive a day prior to anthesis and remains up to 2 days after the opening of flower.
- Tobacco is grouped into often cross-pollinated crops as the natural cross pollination ranges from 5-20 percent.

SUGARCANE (*Saccharum officinarum L.*) (2n = 82-124)

Class: Magnoliopsida, **Sub Class:** asterids **Order:** Solanales, **Family:** Solanaceae**, Sub Family**: Poaceae **Tribe:** Andropogoneae, **Genus:** *Saccharum*, **Species:** *officinarum, barberi, spontaneum*

Floral Structure: The sugarcane inflorescence is an open branched panicle, also known as **'arrow'** whose shape, degree of branching and size are highly cultivar specific. The arrow consists of a main axis and first, second and third order branches and ultimately ending in a rachis, which bear the paired spikelets, one of which is sessile and one pedicellate, both the spikelets are identical in shape, size and structure. At the base of each spikelet is a row of silky white hairs called callus hairs, which impart silky appearance. Each spikelet consists of two glumes as large as spikelets, which enclose Lemma I; Lemma II is absent but has one palea alone. There are two lodicules and sugarcane flowers consist of three stamens and a single carpel with a feathery (plumose) stigma typical of wind pollinated flowers. Anther colour varies from bright yellow to purple. Fruit is dry caryopsis and is very small.

Floral Biology:

- Flowering is dependent on interaction of genotypes and environmental factors such as daylength, temperature and humidity. Some cultivars can flower profusely in their natural environment but flower sparingly when introduced to other regions
- Sugarcane plant flowers only in tropical areas. Warm nights and high humidity favour flowering. Crop planted in Feb-March in South India arrows by the end of October or in the beginning of November. Arrowing is less if the crop is well manured and grown under rich nutrition and irrigation.
- The time of opening of flower also varies very much with variety and season. In general, spikelets open from 3 to 8 am with maximum flower opening between 5-7 am.
- The stigmas protrude first followed by anthers. So, it is slightly protogynous. Sugarcane spikelets open from the top of the panicle, with the outermost spikelets opening first. One to two weeks are required for an arrow to complete flowering.
- Sugarcane is a cross-pollinating species although selfing occurs at low levels.
- Sugarcane seed is often known as **'Fluff'** due to the presence of soft hairs.

FLORAL BIOLOGY OF CROSS-POLLINATED AND OFTEN CROSS-POLLINATED VEGETABLE CROP SPECIES

ONION (*Allium cepa* L.) (2n=16)

Class: Liliopsida, **Sub class:** Liliidae, **Order:** Liliales, **Family:** Liliaceae**, Genus:** *Allium*, **Species:** *cepa*

Floral Structure: Inflorescence of onion is called cume. Inflorescence is present at the top of the green plant which is hollow from the inside. Flowers in the umbel are enclosed in a membranous 2-3 white coloured sheath called spathe. The sheath spilt because of the pressure created by growing flowers inside the umbel. There may be 50-2000 florets in an inflorescence and each individual flower is made up of six stamens, three carples, united with one pistil and six perianth segments. The pistil contains three locules, each containing two ovules. The flowers are white to bluish in colour. Anthers are bilocular and the ovary is superior.

Anthesis/Floral Biology:

- The flower opening takes place in a definitive sequence within the small cymose inflorescence. The anthesis begins from outer flowers and goes centrally in succession.
- Flowering extends to a period of two weeks or more on individual umbel.
- Anthesis occurs in early morning (6-7 am).
- Anther dehiscence takes place between 7:00 am to 5:00 pm and on the next day also.
- Cross pollination occurs as an account of protandry and stigma become receptive when shedding of pollen is over.

SELFING AND CROSSING TECHNIQUES IN CROP PLANTS AND BREEDER'S KIT

Selfing and crossing are the essential procedures in crop improvement process. The exact procedures used to ensure self or cross- pollination of different crops will depend upon the flowering habit and normal pollination behavior of plants. Selfing and crossing are the processes of controlled pollination and the plant breeder shall be expert of these techniques in order to manipulate the pollination of specific plants to achieve the various objectives of crop improvement.

SELFING: In selfing, pollination with pollen of the same flower or the same plant is achieved. Selfing is done with two main objectives of to maintain the genetic purity of a variety and to increase the homozygosity.

CROSSING: In crossing, a flower is pollinated from the pollen of the desired plant. In case of a bisexual flower, we need to avoid its own pollen so that we can pollinate with the pollen from desired plant and removal of anthers or killing of pollen of a flower without affecting the female reproductive organ is known as **emasculation**. In hermaphrodite or bisexual flower, the main purpose of emasculation is to prevent self-pollination. After emasculation flower becomes pistillate type and thus can be crossed with any desired pollen. Crossing is done with the following objectives:

1. To introduce desirable characters of one variety into another variety
2. To create genetic variability or variation
3. To exploit hybrid vigour or heterosis.

SYNCHRONIZATION IN FLOWERING

The principal condition that must be fulfilled in order to bring about the desired crossing, that the partners flower simultaneously. If any of the parent involved in hybridization flower earlier than other, there will be problems in crossing because of stigma is receptive and pollen is not available and vice versa. Therefore, to synchronize the flowering in both the parents the following techniques can be adapted.

- **Staggered Sowing:** The sowing of the parents involved in crossing at a defined interval, In this case, the seeds of late flowering variety will be sown earlier and the seeds of early flowering variety will be after the required interval.
- **Photoperiodic treatment:** Based on light requirement, the crop plants are classified as long day plants, short day plants and day neutral plants and plants needs suitable photoperiod for flowering. If one of the other varieties belongs to the long day period, both the varieties can be made to flower simultaneously either by providing extra light of one-two hour per day through fluorescent lamp to long day variety or by keeping short day plants in darkness for 2-3 hours day during long day condition, respectively.
- **Vernalization:** Seeds of the late flowering variety will be soaked in water so as to initiate germination and then kept in refrigerator for the period equivalent to the difference in flowering between the varieties. Then the germinated seeds are sown along with the early flowering variety. Then the varieties start flowering simultaneously.
- **Pollen storage:** The pollen of early flowering variety may collect and stored in cold storage with saturated carbon dioxide (CO_2) and then may be used for pollination when the late flowering variety begins to flower.
- **Adjusting the cultural operations**: By applying more phosphatic fertilizers or by giving stress such as the late flowering variety may make to flower early by providing less irrigations. In contrast to this, excess application of nitrogenous fertilizers or by giving a greater number of irrigations will delay the flowering in early flowering variety.

SELFING TECHNIQUES IN CROP PLANTS:

- **Bagging:** Inflorescence or flower before it starts blooming with butter paper bag, brown paper bag and cloth bag (two ring three ring bag for mustard) or by any other such means to prevent cross pollination and ensure self-pollination e.g., Paddy, Sorghum, sunflower and mustard.
- **Ringing:** A ring made up of iron is inserted to the tip of the bud to prevent its opening thus enforcing self-pollination e.g., Cotton.
- **Mud smear:** The flower that is expected to open the next day is covered with a thin layer of cotton lint at its tip and the mud is smeared on the lint. The mud gets dried and does not allow the bud to open e.g., cotton.
- **Lantern Method:** Arrow is enclosed in a lantern which is a bamboo frame work covered with closely woven cloth or polythene sheet. The lantern is supported by a bamboo pole. The temperature within the lantern may be high during noon. Therefore, the lantern is removed between noon and 4 pm e.g. Sugarcane.
- **Straw Method:** A short piece of a soda straw which is closed at one end is covered over the stigma, thus ensuring self-pollination e.g. Tobacco.

EMASCULATION TECHNIQUES IN CROP PLANTS :

- **Hand emasculation:** In species with large flowers, removal of anthers is possible with the help of forceps. It is done before anthers dehiscence i.e. generally done between 4 to 6 pm one day before the anther's dehiscence. To avoid the confusion, it is always preferable to remove other young flowers located close to emasculated flowers. The corolla of the selected flower is opened with the help of forceps and the anthers are removed away with the aid of forceps. Sometimes corolla may be totally removed along with epipetalous anthers e.g. Sesame. In all cases, gynoecium should not be injured and anthers should not be remains in the flowers. It is more preferable in such crops where fruit produces a large number of seeds with single like paddy, sorghum, ragi etc. pollination as in case of tomato, brinjal, bhendi, cotton etc. This is also practiced in some of the cereals and millets.
- **Suction method:** Suction method is useful in species with small flowers. Emasculation is done in the morning immediately after the flowers open. A thin rubber or a glass tube attached to a suction hose is used to suck the anthers from the flowers. The amount of suction pressure is used is very crucial which should be sufficient to suck the pollen and anthers but not gynoecium. In this method, considerable self-pollination, up to 10 % is likely to occur. Washing the stigma with jet of water may help in reducing self- pollination; however, self-pollination cannot be eliminated completely in this method, e.g., Rice.
- **Hot water treatment:** Pollen grains are more sensitive than female reproductive organs to both genetic and environmental factors. Hot water has been used to kill the pollen in sorghum, rice and grasses and thus removal of anthers is avoided. The flowers are immersed in hot water with temperature ranging from 42 to 48^{o}C with period varying from one to ten minutes depending upon the species e.g., for rice 10 minutes with 40 to 44 ^{o}C is adequate. Treatment is given before the anther's dehiscence and prior to flower opening. Hot water is generally carried in thermos flask and whole inflorescence is immersed in hot water.
- **Cold water treatment:** Chilling is employed in wheat and rice with temperature around freezing which kills the pollen grains without affecting gynoecium e.g., in case of rice, treatment with cold water 0.6^{o}C. This is less effective than hot water treatment.
- **Alcohol Treatment:** It is not commonly used. The method consists of immersing the inflorescence in alcohol of

suitable concentration for a desired period followed by rinsing with water. Pollen of lucerne can be killed by immersing the flower in 57% Ethyl alcohol for a period of ten minutes.

- **Removal of male flowers:** In monoecious plants, male and female flowers are separate and present on the same plant e.g., maize, castor and cucurbits. Male flowers from the female parent are removed and the female flowers are either hand pollinated or allowed to be pollinated by wind. At the time of harvesting, seeds from the female parent are collected.
- **Removal of male plants:** In dioecious plants such as spinach, asparagus and papaya the male plants usually flower earlier. These male plants near the female parents are removed and then pollinated with desired pollen.
- **Chemical hybridizing agents (CHA):** Spraying of solutions of malic hydrazide (MH) (200 ppm) or NAA (450 ppm) on seedlings selectively kill the male gametes without affecting female gametes e.g., ethrel, sodium methyl arsenate, zinc methyl arsenate in rice, MH for cotton and wheat and Gibberellic acid spraying in sunflower (star bud stage) before flower opening.
- **Genetic emasculation:** Genetic/cytoplasmic male sterility may be used to eliminate the process of emasculation. This is very useful in commercial hybrid seed production of rice, sorghum, cotton, maize, mustard, pearl millet and onion etc. In self-incompatible cases, emasculation is not necessary, because self-fertilization will not take place. Protogyny (pearlmillet) and protandry (maize) also facilitate crossing without emasculation.

Precautions should be taken during emasculation:

- Select appropriate healthy bud.
- Don't injure other parts of flower.
- Use of envelopes usually after emasculation just to protect the bud from foreign pollen.
- Remove too young and too mature buds.
- No stamen should remain in flowers and check the stigma is free from pollen.
- Generally, emasculation should be done in the evening, because stigma will not be receptive in the evening and injury will heel up in the night.
- Proper tagging is necessary for emasculation.

POLLINATION METHODS: Pollination must be made during the period when the stigma is receptive. It is carried out by collecting ripe anthers which may be brushed and the pollen is dusted on ovary or rubbed on stigmas by means of forceps or camel hair brush. In some cases, the pollen shedding panicle can be shaken over the emasculated florets. It is essential that the pollen be matured and fresh.

SPECIAL TECHNIQUES OF CROSSING:

- **Approach /Contact Method:** In this method, the plants that are to be hybridized are grown side by side and at flowering they are brought together in a paper bag to enable cross pollination. The male parent used in this method must carry a gene marker to identify the crossed F_1. This method is usually followed in the crops with miniature flowering which are difficult for emasculation and pollination e.g. ragi.
- **Test tube method:** A wide test tube or small flask lined with moist filter paper is inserted over the inflorescence and plugged with cotton. Due to the high humidity developed inside the tube the anthers emerge without shedding / bursting and may be cut off easily and then pollinated with desired pollen e.g., ragi.
- **Humphery and Tuller method:** Here only a few anthers near the stigma are removed and a soda straw is inserted over the stigma and pushed down. In this operation, the remaining anthers get cut off by the straw during insertion. A small quantity of stamen from the male parent is scooped into the straw and then inserted over the stigma and pushed down till the end reaches the ovary. The free end is bent and closed and the bracts are pulled up around the straw and wire securely to hold it in place. Thus, in this process emasculation and pollination are done simultaneously e.g., Cotton.
- **Shivashankar's method:** This method is followed in pulse crops. On the evening previous to the day of pollen shedding, transverse cut is made in flower bud such a way to facilitate removal of upper portion of the corolla like a cap without causing injury to the gynoecium. During this process, the anthers are clipped off automatically and remain inside the corolla cap which is removed. The stigma is then pollinated with desired pollen on the next day morning.
- **Male sterility:** Male sterility is a condition in which pollen is absent or non-functional. There are three types of male sterility.
  1. **Genetic Male Sterility:** Here nuclear genes condition the male sterility either in recessive or in dominant condition e.g., pigeon pea, sesame, sunflower.
  2. **Cytoplasmic Male Sterility:** It is controlled by the action of cytoplasm. Since the cytoplasm is transmitted through the female gamete only. So, it is inherited and transferred by the female parent. This type of sterility is commercially feasible in crop plants where vegetative parts have commercial value like onion, potato and ornamental plants.
  3. **Cytoplasmic Genetic Male Sterility:** It is due to interaction of cytoplasmic and genetic factors. It differs from cytoplasmic male sterility in that single dominant nuclear gene for fertility can overcome the effect of cytoplasm responsible for sterility e.g., rice, maize, sorghum, pearl millet, wheat, cotton.

Presence of male sterility eliminates the emasculation process. This has been made commercial production of hybrid seeds in sorghum, bajra, sunflower and onion.

BREEDER'S KIT/ INSTRUMENTS REQUIRED

S. No.	Items	Uses
1	Fine Pointed straight or curved scissors	To remove unwanted buds, awns etc.
2	Pointed Forceps	Required for emasculation
3	Magnifying lens or Hand lens	To observe small flowers and different floral parts etc. (stigmatic surface, dehiscence of anthers)
4	Needles	To open small buds and separating the floral parts
5	Small camel hair brush	Camel hair brushes of size 3 0r 4 for collection of pollen and transfer of pollen to stigma
6	Bags	Parchment or butter paper bag, muslin cloth bag and paper bags of different sizes for different crops
7	Petri dishes	Required for collecting pollen grains
8	Alcohol	A small vial of alcohol to sterilize forceps, scissors, needles, petri dishes etc.
9	Tags/Labels	Field labels (paper, card board or aluminum tags) of suitable sizes for labeling the plants
10	Field Stool	For emasculation and pollination
11	Meter Scale	Required for plant measurement in the field
12	Field note books and pencils	Required to note down observations in field
13	Gem Clips and rubber bends	For bagging and tagging

MALE STERILTY SYSTEM IN HYBRID SEED PRODUCTION OF FIELD CROPS

The term sterility covers those cases of infertility or barrenness resulting from irregularities in the sexual reproductive system. Infertility may be caused by abnormal or imperfect development of the reproductive organs. The stamen or pistil may be real formed, the pollen defective, or the ovules aborted. When sterility is due to the failure of functional anthers or pollen, it is termed male sterility. Female sterility is failure to produce functional ovaries or eggs. Generally, female sterility systems have been less stable and dependable than male sterility systems.

In case of genetic male sterility, we find normal and comparing of homologous chromosomes and the sterility is due to gene effects. Even though the sterility is disadvantageous as an agriculturist, seed is food and failure in seed set is mainly due to the factors which affect fertility and results in sterility. But it can be used advantageously in inducing male sterility which facilitates easy crossing. In male sterile plants, flowers do not produce functional anthers or viable pollen, but ovaries function normally. Although the flowers cannot be self-pollinated, they can be cross-pollinated. Hand emasculation in bisexual flowers which are very minute in size is a very tedious and cumbersome job as it consumes more time and resources. If male sterility is induced genetically in crop plants, it saves time and resources and reduces the cost of production of hybrid seeds.

Male sterility may be conditioned due to cytoplasmic or nuclear genes, or due to interaction of both. Depending on these factors, it is classified as below:

1. Cytoplasmic Male Sterility (CMS)
2. Genetic Male Sterility:
   a) Environment insensitive
   b) Environment sensitive - Temperature sensitive Genetic Male Sterility (TGMS)
   Photoperiod sensitive Genetic Male Sterility (PGMS)
3. Cytoplasmic–Genetic Male Sterility (CGMS)
4. Transgenic Male sterility

COMPONENT LINES OF MALE STERILITY SYSTEMS FOR HYBRID SEED PRODUCTION:

A line / Male sterile (ms) line: It is a **male sterile line** belonging to any of the above male sterility system. By virtue of their nature, A line is always used as female / seed parent in hybrid seed production.

B line / Maintainer line: Maintainer or 'B' line is an isogenic line to 'A' line except for fertility status. It is used to **maintain the sterility of A** line. On crossing A line x B line (ms line x maintainer line), viable and normal hybrid seeds are formed, but the F_1 plants developed from these seeds fail to produce viable pollen grains and thus remain sterile. These sterile F_1 progenies will again produce hybrid seeds as above on crossing with B-line, and so forth. For maintenance of sterility the characteristic features of A-line must remain constant or unaltered generation after generation on crossing with B-line.

Maintenance of sterility signifies three points:

(i) Viable hybrid seeds are produced by A x B crosses
(ii) These hybrid seeds produce sterile progenies which can be maintained by re-crossing with B-line,
(iii) The characteristic features of A-line are maintained over generations since both A and B lines are isogenic.

R line or Fertility Restorer line: 'R' line restores the fertility in 'A' line by crossing 'A' line x 'R' line and the resultant F_1 hybrid seeds which is of commercial value. Hybrid seeds give rise to F_1 progenies, which also produce viable pollen grains and normal (F_2) seeds in farmer's fields. However, when F_2 seeds are re-sown, the male sterile plants will reappear as a

consequence of segregation. This makes them useless for further cultivation in the farmer's field. Fertility restoration can be done either by a fertile cytoplasm or nuclear genes on chromosome. Since, A x R progenies are of commercial significance, the choice of R-lines should be such that they produce highly heterotic and disease-free hybrids.

DIFFERENT MALE STERILITY SYSTEM FOR SEED PRODUCTION

Genetic Male sterility (GMS): GMS is manifested through the action of nuclear genes inhibiting normal development of anthers and pollen. The precise stage at which pollen development is interrupted may differ with the species, or with the specific male sterility gene. Genetic male sterility is predominantly conditioned by a recessive allele, *ms.* The dominant *Ms,* resulted in production of viable pollen. For the diploid species, the homozygous recessive (*ms ms*) would be male sterile while homozygous dominant and heterozygous dominant genotypes (*Ms ms and MsMs)* would be male fertile. The seeds harvested from male-sterile plants (*ms ms*) may be pollinated by either homozygous (*Ms Ms*) or heterozygous (*Ms ms*) male-fertile plants. If pollination is by the latter, the progeny will segregate 50% *Ms ms*: 50% *ms ms*. If a male-sterile plant (*ms ms*) is pollinated by a homozygous male-fertile plant (*MsMs*), all F_1 plants will be heterozygous and male-fertile (*Msms*) as noted above, but the F_2 generation will segregate 25% *Ms Ms*: 50% *Ms ms*: 25% *ms ms*. Male sterility genes have been identified in barley, corn, cotton, flax, pearl millet, potato, rice, sorghum, soybean, tobacco, wheat and other crops. **Genetic male sterility** is subdivided into 2 broad groups:

A) Environmental insensitive - *ms* gene expression is not much affected by the environment.

B) Environmental sensitive- *ms* gene expression occurs within a specified range of temperature and/or photoperiod regimes; this type of sterility is known in rice, tomato, wheat. Environment sensitive male sterility is further divided into two groups:

i) **Temperature Sensitive Genetic Male Sterility (TGMS):** Thermosensitive Genic Male Sterile lines are genic male sterile lines whose male sterility/fertility alteration is conditioned by different temperature regimes. For example, most of the TGMS lines remain male sterile at a high temperature (maximum >30°C) and they revert back to partial fertility at a lower temperature (maximum <30°C). The critical sterility/fertility points (e.g., 23.3°C or higher for rice TGMS line Pei-Ai645), vary from genotype to genotype. The critical thermosensitive stage for fertility alteration in the TGMS line varies from formation of PMC's to meiosis i.e., 15 to 25 days before heading or 5-15 days after panicle initiation. This type of genetic male sterility is being used to develop hybrid rice in China.

ii) **Photoperiod Sensitive Genetic Male Sterility (PGMS):** Photoperiod sensitive genic male sterility includes genic male sterile lines which respond to the photoperiod or duration of day length for expression of pollen sterility and fertility behavior. For example, most of the PGMS lines remain male sterile under a long-day (>13.75h) conditions and revert back to fertility under short-day (<13.75h) conditions. The stage of development sensitive to photoperiod lasts from differentiation of secondary rachis branches to the formation of PMC's. It is important that a temperature above the critical range (23-29°C for rice PGMS) leads to male sterility under any photoperiod, while those below the critical range produce male fertility irrespective of the photoperiod.

Cytoplasmic Male Sterility (CMS): Cytoplasmic male sterility is determined by the cytoplasmic genes (mitochondrial and chloroplast genes), but may be influenced by nuclear genes. Like genetic male sterility, it results in the production of flowers with nonfunctional anthers or pollen. Cytoplasm that causes an organism to be male sterile may be referred to as sterile cytoplasm (S) or (CMS), in contrast to normal cytoplasm (N), which permits normal development of functional anthers and pollen. The sterile cytoplasm often results from the introduction of nuclear chromosomes into a foreign cytoplasm. For example, cytoplasmic male sterility in sorghum was obtained by transferring kafir chromosomes into cytoplasm of milo. Male sterility is not expressed when the milo chromosomes are in milo cytoplasm. The kafir chromosomes were introduced into milo cytoplasm by pollination of a milo plant with kafir pollen, and successively crossing the progeny as the female back to kafir as the male, until the entire set of kafir chromosomes was recovered.

Progenies from F_1 seeds would be always male sterile plant since their cytoplasm transmitted only through the female parent. CMS is not influenced by environmental factors (temperature and photoperiod) so it is stable.

Uses: CMS can be used in hybrid seed production of certain ornamental species or in species where a vegetative part is of economic value. This type of male sterility found in onion, fodder jowar, cabbage, sugar beets etc.

Disadvantage: CMS is not useful for crop plants where seed is the economic part because the hybrid progeny would be male sterile.

Cytoplasmic Genetic Male Sterility (CGMS): CGMS is also known as **nucleoplasmic male sterility.** CGMS only differs from CMS that the nuclear gene (R) for fertility restoration is known for the respective male sterility gene (ms). Restorer gene (R) is generally dominant and presence of a dominant fertility-restoring allele, the sterile cytoplasm becomes inoperative and the anther produces normal viable pollen; in the presence of the contrasting recessive alleles, male sterility is expressed. In hybrid seed production, the parent with the sterile cytoplasm necessarily is used as the female (A line, S, rr) and the fertility-restoring genes are contributed by the male parent (R line, S/N, RR or S/N, Rr). Maintainer line (B line) is the isogenic line to male sterile line (A line, S, rr) except normal cytoplasm and 'A' line will be maintained by crossing with 'B' line.

Chemically Induced Male Sterility: Chemically induced male sterility offers the breeder a non-genetic alternative of inducing male sterility with the use of chemicals called gametocides, pollen suppressants, pollenocide, androcide and

chemical hybridizing agents (CHA). This method is very useful for plants with bisexual flowers in which it is difficult to obtain genetic or cytoplasmic-genetic male sterility. In this method of developing hybrids, the foliar spray of CHAs can be effectively used on female parents prior to flowering that can kill pollen grains of treated plants without affecting pistillate reproductive organs or seed development. A major problem has been failure to obtain complete pollen sterility, due to variations in response with different genotypes of the crop, environmental effects on the action of the chemical or different effects of the chemical itself. Chemically induced male sterility is used in cotton, wheat, rice, sorghum and other crops including vegetables but varying degrees of response on male sterility has been observed.

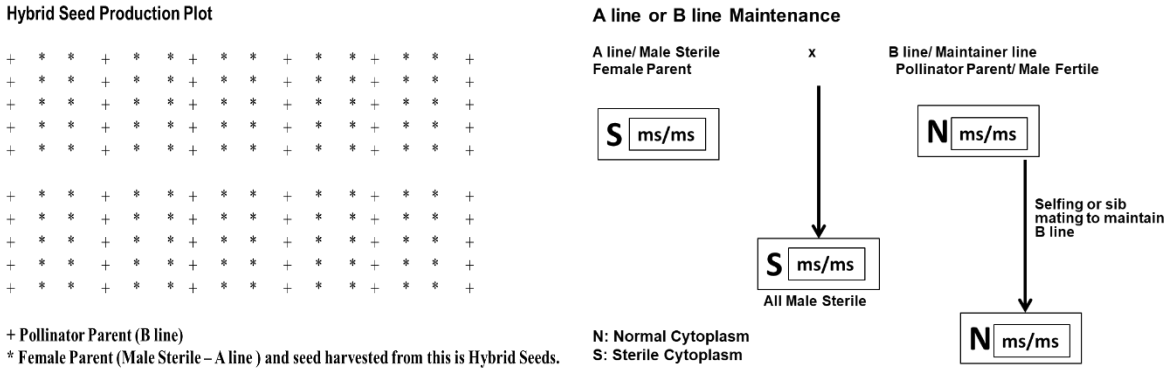
Transgenic Male Sterility: Recombinant DNA techniques or genetic engineering for disturbing any developmental steps required for the production of functional pollen within the microspore or for the developmental of any somatic tissues supporting the microspores. Many transgenes have been shown to produce genetic male sterility, which is dominant to fertility. Consequently, it is essential to develop effective fertility restoration system if these are to be utilized for hybrid seed production. An effective restoration system is available in at least one case called Barnase or Barstar system. The Barnase gene of *Bacillus amyloliquefaciens* encodes extracellular RNAase and it is expressed only in tapetum cells causing their degeneration. Transgenic tobacco and *Brassica napus* plants expressing Barnase were completely male sterile. Another gene, Barstar, from the same bacterium encodes a protein, which is a highly specific inhibitor of Barnase and dominant over the barnase gene. Therefore, transgenic plants expressing both Barstar and Barnase are fully male fertile. The Barnase gene has been tagged with bar- gene, which specifies resistance to the herbicide, phosphinothricin. This male sterile line is maintained by crossing with a male fertile line. The progeny so obtained contain 1 male sterile : 1 male fertile plants; the latter are easily eliminated at seedling stage by a phosphinothricin spray. Cross male sterile (barnase) with male fertile (barstar) to get hybrid seed, which now has both barnase and barstar expressed in tapetum and, hence, is fully fertile. This system of male sterility is yet to be commercially used.

LEARNING TECHNIQUES IN HYBRID SEED PRODUCTION USING MALE STERILITY IN FIELD CROPS

Heterosis breeding may be regarded as most spectacular phenomenon of crop breeding in last century which witnessed not only the increase in productivity of crops like maize, pearl millet and sorghum but also the application of hybrid technology in self-fertilized crops predominantly in rice. The earlier concepts of exploiting the heterosis in cross pollinated crops changed, mainly due to success of hybrid technology in rice. Male sterility is unique gift of nature to mankind and it is of special interest of plant breeders to produce more efficient and economic hybrid seeds. Enhancement of productivity has been an important goal for most breeding programs. Although the hybrid breeding was known as a potential way of yield increase, but the constraint of large- scale hybrid seed production prevented the commercialization of this technology in many crops. Large scale hybrid production became possible by the use different kinds of male sterility systems and it helps in combating global hunger through its use in developing high-yielding hybrids in various food crops.

Hybrid Seed Production using CMS: The basic method of hybrid seed production using a cytoplasmic male sterile parent is illustrated in **Fig.** given below. Cytoplasmic male sterility can be utilized for producing hybrid seeds in those vegetables or ornamentals where the vegetative part is of commercial value (e.g., onion, carrot, radish, cole crops etc.).

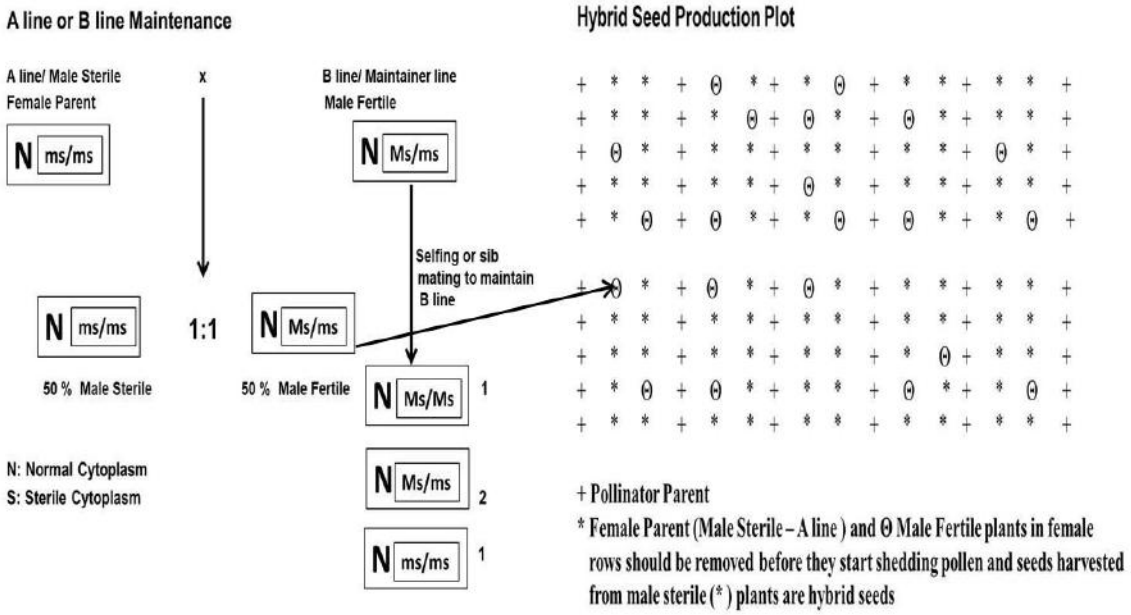
Maintenance of lines: Cytoplasmic male sterility can be maintained by crossing a male sterile line (A line) with the maintainer line (B line) which is used as the recurrent parent in the backcross programme during male sterile development since the nuclear genotype of the pollinator (B line) is identical to that of the new male sterile line. Male sterile Male sterile line maintenance is presented here.



Hybrid seed production using cytoplasmic male sterility system

Hybrid Seed Production Using GMS (Two-line System): The first step in practical deployment of the GMS system is seed multiplication of male sterile (A line) and seed multiplication of A line is not possible through selfing or sib mating, since pollen are not functional or sterile pollen grains. The hybrid seed production with genetic male sterility lines is very tricky and

requires greater attention and resources. Commonly utilized homozygous recessive male sterile line (ms/ms) is maintained by back crossing it with heterozygous isogenic line (Ms/ms) for male sterility or maintainer line (B line) in isolation. Therefore, in the hybrid seed production field, female line would contain 50 % male sterile (ms/ms) and 50% male fertile segregants (Ms/ms); latter must be identified and removed before pollen shedding. Hence, the stage of identification of male sterile/fertile plants in the hybrid-seed production field is very important. Identification of male fertile is done in seedling stage either due to pleiotropic effect of the ms genes or due to the phenotypic effect of closely linked marker genes. Thus, labour and the time required to identify and remove fertile plants in the hybrid-seed production field can be avoided. The removal of fertile plants before transplanting also facilitates proper production management, especially the maintenance of the female plant (male sterility) population per unit area increases productivity of F_1 seeds. The productivity of hybrid seeds can be increased by two ways: i) Removal of male fertile plants before transplanting which increases the female plant (A line) population per unit area and ii) male line (pollinator) should good pollen shedders to achieve satisfactory seed set in the female line. Scheme of hybrid seed production with the genetic male sterility is illustrated in second figure.

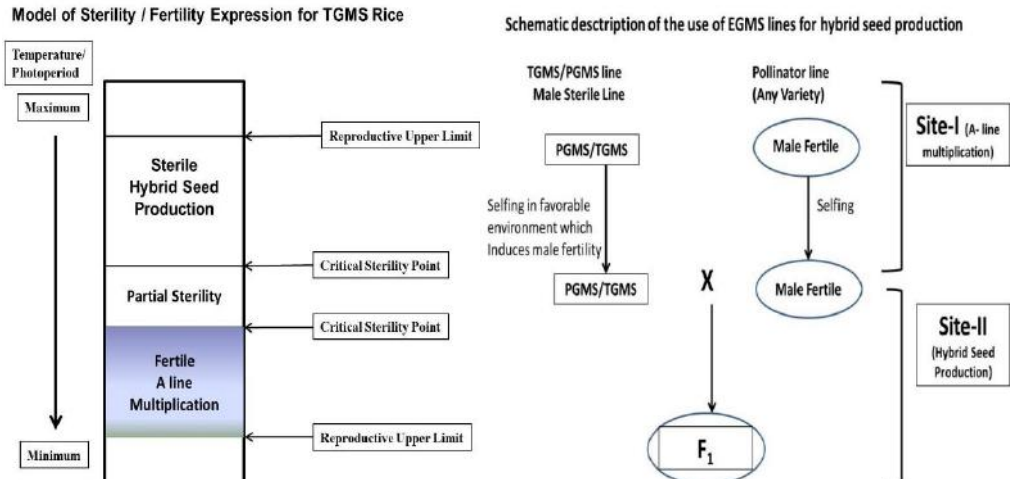


Hybrid seed production system using genetic male sterility

Hybrid Seed Production using Environment Sensitive Male Sterility-EGMS (TGMS and PGMS): The seed production system involving environment-sensitive line is fascinating and selection of sites for hybrid seed production and multiplication of different lines is critical. In this system two different sites with distinct and stable climatic parameters (temperature or photoperiod) are required during crop growth essential. Unlike the CMS system, seed production in EGMS system is relatively easy as no maintainer line is required for multiplication of EGMS (TGMS or PGMS) line. Only TGMS or PGMS line and pollen parent are needed to produce a hybrid. Hence, the hybrids developed by using PGMS or TGMS system are called 'Two line' hybrids.

Male sterility maintenance site: For the maintenance of male-sterile line, its seed are multiplied by selfing like any other varieties when they are grown under an environment where favorable temperature or photoperiod is present under which male fertility is induced and all the plants should be male fertile.

Hybrid seed production site: Crop should grow at a site which has critical temperature or photoperiod under which the male sterility of the line will be maintained. In



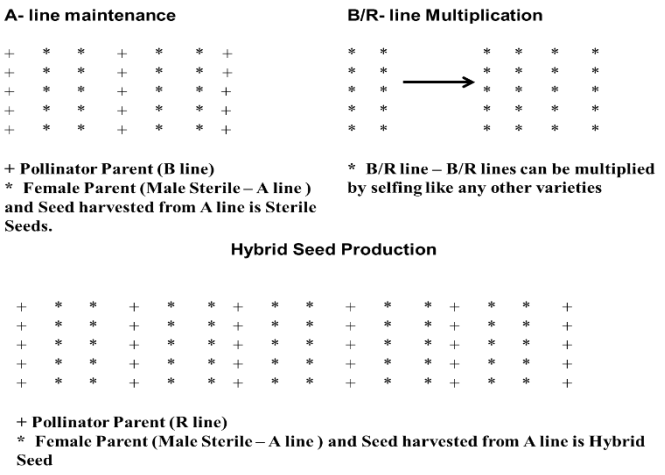
Two-line hybrid seed production system using EGMS (TGMS/PGMS) system

this site all the plants will be male sterile and these are used for hybrid seed production. The male and female lines are grown in specific ratio and the hybrid seeds are harvested from the male sterile lines.

Hybrid Seed Production Using CGMS: This is the case of cytoplasmic male sterility where a nuclear gene for fertility restoration (*Rf*) in the male sterile line is known. Hybrid seed production based on CGMS (CMS-FR) system requires four isolations:

Maintenance of CMS line (A line): A x B, by growing of A line and its corresponding B line together in an isolated plot with fixed male and female row ratio. Pollen produced by B line will fertilize the male sterile plants (A line) and seeds produced thus; seeds harvested from A line will give rise to A line seeds again.

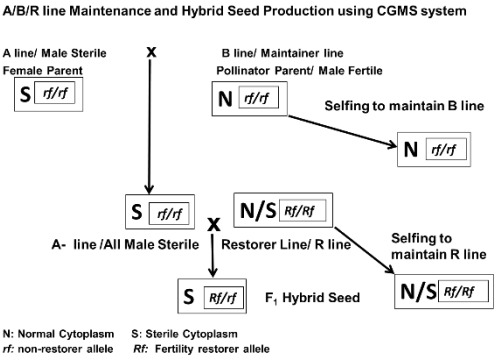
Multiplication of maintainer line (B line): B line can be multiplied like any other variety through selfing. Purity of B line is important because it is used to maintain the parental line (A line) of hybrids.



Layout for isolation plots for component line maintenance and Hybrid seed production under CGMS system

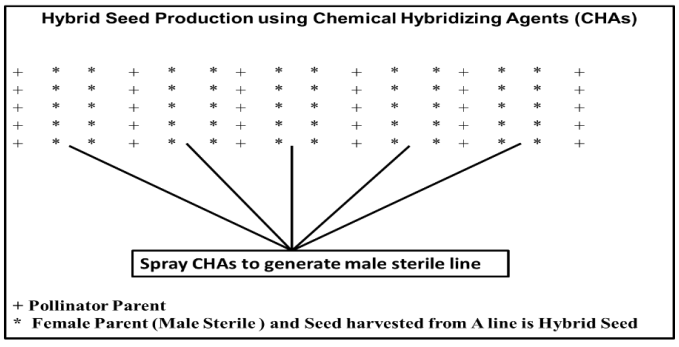
Multiplication of restorer line (R line): Seeds of R lines are multiplied in isolation like any other variety i.e. through selfing. Any plant in the R line plot appearing different from true R type should be rogued out before anthesis because purity of the parental seed is very important because it affects the quality of hybrid seeds that is generated.

Hybrid seed production (A x R): For hybrid seed production, fixed no. of rows of A line (S *rf/rf*) are alternated with sufficient no. of R lines (N *Rf/Rf*) which can provide enough pollen to A lines.



Hybrid Seed Production Using CHA's: A chemical with the ability to induce pollen sterility or check pollen shed can not only help to overcome problems associated with the CMS but will also impart greater flexibility in choice of genotype as potential female parent in the development of hybrids. In this method of developing hybrids, male sterility is induced by spraying a variety with chemical gametocide that can kill pollen grains of treated plants without affecting the pistil and it acts as male sterile line (female). In hybrid seed production, two parents are planted in alternate strips. One is sprayed with chemicals at appropriate growth stage, and the other is used as pollen source to produce the hybrid seed.

Hybrid Seed Production Using Transgenic Male Sterility (Barnase/Barster) System: The first success in developing genetically engineered male sterility in crop plants was by transforming tobacco and rapeseed plants with dominant gene barnase (bacterial RNase) driven by a tapetum-specific promoter TA 2. The barstar gene has been used to prevent the activity of barnase gene leading to male fertility of plants carrying both of the genes, i.e., the barstar gene can be used as a dominant gene for fertility restoration. Barster gene is dominant over the Barnase. To get the hybrid seed, male sterile (barnase) is crossed with male fertile line (barster) and hybrid seed will have both barnase and barster gene expressed in tapetum and hence, hybrid seed is fully fertile.

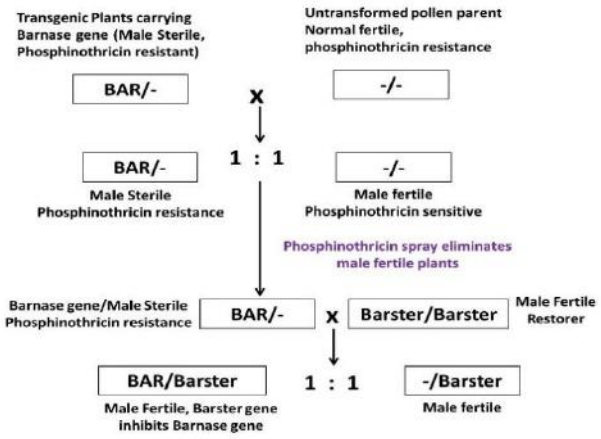


Hybrid seed production using CGMS system- A hereditarily illustration

Practical Activities:

1. Visit the mustard hybrid seed production field using CGMS system and isolation plots for 'A' line multiplication.
2. Collect fresh flowers from 'A' line which is male sterile, 'B' line which is male fertile and 'R' line which is a fertility restorer line and is male fertile.
3. Collect pollen dust from fresh flowers of different A, B and R lines in petriplates by using brush.
4. Apply small quantity of pollen dust from respective lines on different slides.
5. Prepare 2% Acetocarmine stain solution and apply one or two drops on each of the microslide containing pollen dust and place a micro-coverslip on it.
6. Observe each slide under the microscope and count the stained and unstained pollens.

7. The stained pollen grains are fertile ones and unstained pollens are sterile. Count the number of fertile pollen grains in each microscopic field and record the results of 10 microscopic fields for each B and R line and express the fertile pollen grains in terms of %.
8. Pollen fertility (%) = [No. of stained pollen grains / Total no. of pollen grains] x 100



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Hybrid seed production using Barnase/ Barster system- A hereditarily illustration

OPTIMIZATION OF HYBRID SEED PRODUCTION

Commercial hybrids have the greatest potential for crops in which the hybrid seed can be produced reliably and economically. Three biological requirements for successful hybrid seed production include the presence of hybrid vigor, elimination of fertile pollen in the female parent and adequate pollination by the male parent. If the biological requirements have been met in a species, a practical program of seed production on a large scale must be developed before hybrids can be used by farmers. The seed production cost for hybrids is usually higher than that of varieties. Even though, the seed producers follow defective production practices like improper planting ratio of male and female rows, non-synchronization in flowering between female and male parents and incomplete rouging in female lines. Hence, it is essential to undertake the following points in to account for optimization of the hybrid seed production programme:

Genetic Purity of Parental Lines: A basic requirement for commercial hybrid production is an adequate supply of pure seed (foundation seed) of the both the male and female parents. Quality foundation seed production is possible by securing a pure seed of the parental lines from the plant breeder and to maintain a high degree of purity during its multiplication. Production of pure seed of parental lines depends on careful selection of field, isolation of afield, rouging off-types plant and male fertile plants from female parents and careful harvesting and seed conditioning. Genetic purity of parental lines is critical because it reduces the cost of rouging in large hybrid seed fields. The types of parents that must be increased as foundation seed depend on the male sterility system such as, Inbred lines in conventional hybrid seed production, In CGMS system: A, B and R lines and GMS: A and B lines.

Field Selection: Land selected for hybrid seed production should be fertile, protected irrigation and drainage system, enough sunlight, devoid of weed infestation and volunteer plants (Volunteer plants are the unwanted plants growing in the seed production field from the previous seasons' crop) and No serious disease and insect problems.

Isolation: Proper isolation is necessary to ensure genetic purity of hybrid seed and isolation requires from the sources of pollen from other lines, hybrids or weeds that can cause unwanted cross pollination. Isolation is required in multiplication of parental lines as well as in production of hybrid seed. Isolation distance varies from crop to crop and it can be achieved by following ways:

Space isolation: No other varieties or hybrids should be grown except pollinator parent.
Time isolation: a time of flowering of female and male parent may vary from other variety or hybrids.
Barrier/Physical isolation: topographic, tall crops and artificial obstacles can be used to avoid contamination

Row Ratio and Row Direction: Row ratio refers to the ratio of number of rows of the male parent to the female parent. The female to male ratio depends largely on the abundance and duration of pollen production by the male parent. Greater proportion of female rows is desirable to enhance the production of hybrid seed, care must be taken to ensure adequate pollen supply to facilitate hybrid seed set on female plants. Female to male ratios for sorghum vary from 3:1 to 6:1, maize from 2:1 to 4:1, sunflower from 2:1 to 7:1, mustard 2: 4 to 2: 16 and rice 2:4 to 2:8. Row direction perpendicular to the prevailing wind direction at flowering stage allows easy pollen dispersal on the seed parent. The layout of row ratio depends on: i) growth duration of the male parent ii) amount of pollen shed and iii) height of the male line.

Rouging: In the hybrid seed production technology rouging is a crucial operation to be followed from field to storage. It is the process of removal of off types like other varieties, weeds and fertile plants in female parent. The removal of off-type plants from both the female and male parents is necessary to maintain the genetic purity of hybrid seed. Female parents with cytoplasmic-genetic male sterility must be rouged for male –fertile plants before pollen is produced. The frequency of rouging is determined by the number of off-types and rate of development of flowering and rouging should not be done in high sunshine condition.

Synchronization in Flowering: Synchronization in flowering of male and female parents is essential to increase seed set and may require manipulation in planting or during growing season. Parental lines differ in flowering date should be avoided in hybrid combinations whenever possible because hybrid seed production becomes more complicated, costly and unreliable. Planting parents on different dates, commonly referred as delayed, split or staggered sowing, is the most common procedure to achieve synchronize in flowering for optimum seed set. Crop specific cultural manipulations or by application of selective growth regulators to delay or advance flowering in male/ female lines is also suggested to bring synchronization. One or more technique to synchronize the flowering can be adopted and choice depends on effectiveness and economics of the technique. Generally, treatments should be applied to male parents that will avoid loss of seed production from female lines.

Supplementary Pollination: Supplementary pollination is the increase in pollination of the female parent through artificial means to serve the purpose of high percentage of seed set. There are several ways to supplement pollination in different crops like rope pulling in rice, rubbing heads in sunflower, supplementary hand pollination in fruits and vegetables and honey bee hives in case of mustard, sunflower etc. Supplementary pollination also caters the need of increase in fruit set in vegetable and fruit crops.

ROLE OF POLLINATORS IN HYBRID SEED PRODUCTION

Pollination: The transfer of pollen grains from the anthers to stigma of the pistil of flowers.

Pollinators: An organism (insects, bats, animals, wasps etc.) that transfers pollen from the anthers to the stigmas of flowers, thus effecting pollination.

Pollination is one of the most important mechanisms in the maintenance and promotion of biodiversity and, in general life on earth. Many ecosystems, including many agro-ecosystems, depend on pollinator diversity to maintain overall biological diversity. Pollinators strongly influence ecological relationships, ecosystem conservation and stability, genetic variation in the plant community, floral diversity, specialization and evolution. Pollination also benefits society by increasing food security and improving livelihoods. It is known fact that only where pollinators lived could plants evolve a mechanism of reproduction that involved pollination.

Pollinators are extremely diverse, with more than 20,000 pollinating bee species and numerous other insects and vertebrate pollinators. Organisms that are known to be good pollinators of different crops, vegetables and ornamental plants include bees, butterflies, humming birds, moths, wasps, nectar feeding bats and animals. Bee and wind is common pollinating agents in cross pollinated crops. Some crop species having heavy and sticky pollen grains so cannot be carried by wind and pollination is possible due to only insects or animals. As we see, honey bees became the ideal and mostly the only pollinators as it is almost impossible to manage other insects for planned pollination. Bees provision their nests with nectar and pollen. They methodologically and rapidly go from flower to flower in collecting their food, and in no way injure the flowers. The body of the bee is coated with branched or plumose hairs to which the pollen clings and is transported from flower to flower. Approximately 75 percent of the crop plants grown worldwide for food, beverages, condiments, spices and medicines are pollinated by pollinators.

Pollinators is a critical input for hybrid seed production of maximum cross-pollinated crops like sunflower, mustard, cabbage, carrot, radish etc. In crops like sunflower and cotton pollen is heavy and sticky so cannot be carried by wind. Therefore, pollen is transferred from male parental lines to female parental lines by insects only. In many of the crops hybrid seed is produced by using cytoplasmic male sterility system. In which CMS line (A line) is pollinated with maintainer line (B line) for its maintenance and with restorer line (R line) for hybrid seed production. In general, pollinators visit 'R' line more frequently compared to 'A' line. The augmentation of honey bee hives in the seed production field during flowering period is advisable to increase seed setting and seed yield by supplementing the open pollination. These pollinating agents when used in male sterile hybrid seed production enable us to significantly reduce the cost of hybrid. Therefore, the introduction of honeybees on agricultural crops, especially in hybrid seed production, is an alternative to increase hybrid seed production and increase in yield is due to more activity and foraging behavior of colonies.

Estimates of increased seed set due to pollinators in different crops:

Crop	Percentage
Radish	22-100
Cabbage	100-300
Turnip	100-125
Carrot	91-135
Onion	345-500
Sunflower	130-180
Cotton	70-100
Rapeseed	75-145

Recommendation of bee hives in various important crops:

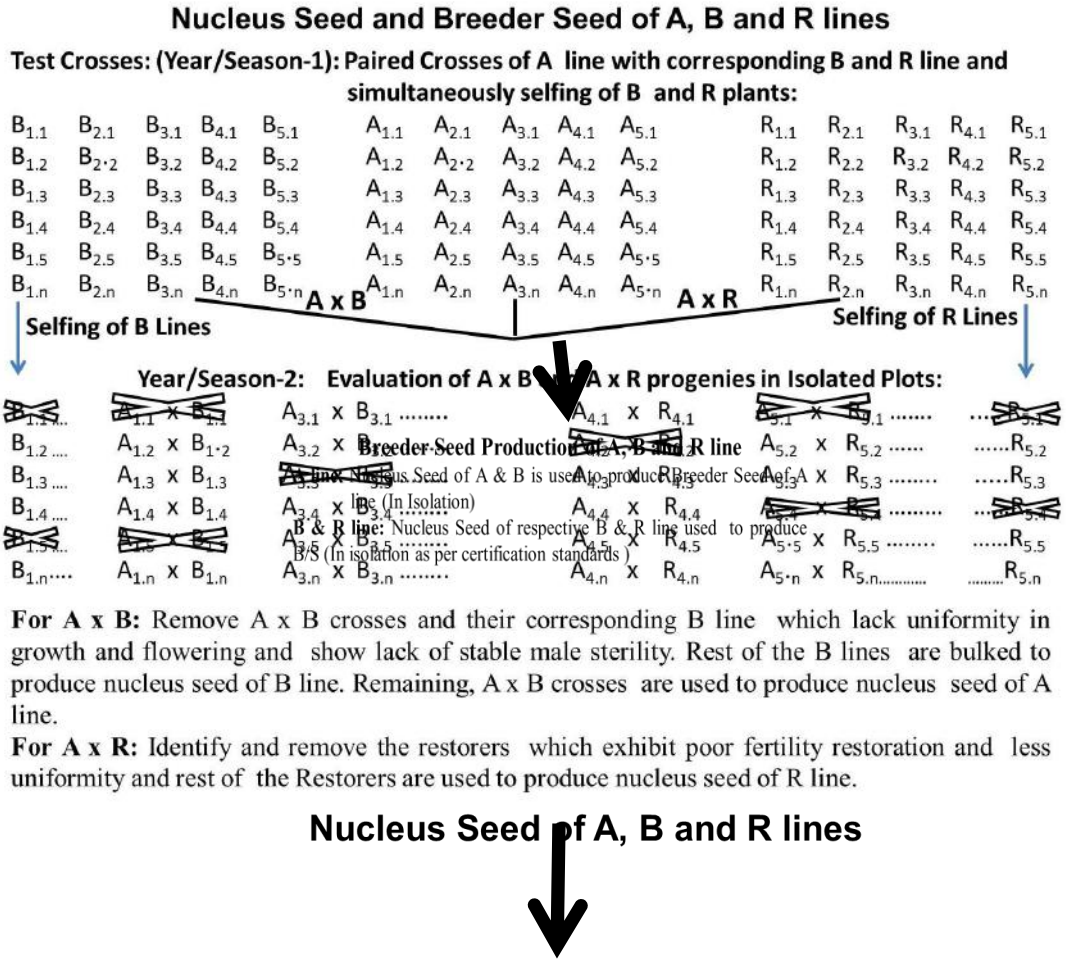
Crop	No. of bee hives per hectare
Alfa-alfa	2.5-8.0
Rapeseed	2.5-15
Cucumis melo	2.5
Carrot	5.0
Cotton	2.5-5.0
Cucumber	10
Onion	10-25
Soybean	1 – 1.5
Gourds	2.5- 5.0
Sunflower	4.0
Radish	5.0

A/B/R LINE PURIFICATION AND DIFFICULTIES IN HYBRID SEED PRODUCTION

The hybrid seed is a product of the two parents. Therefore, it becomes essential that the seed of parental lines is of genetically pure and good quality to exploit the full potential of the hybrids. In hybrid breeding programme, maintenance of genetic purity of parental lines is of prime importance for the development of true hybrid seeds. Lack of purity in parental lines and improper isolation distance in seed production are the major causes of poor hybrid seed quality. Complex nature of fertility restoration has been observed in some restorer line because the restoration lines have not been bred for fertility restoration. With differential restoration, the F_1 progenies will show segregation for fertility and ultimately heterosis level of the hybrid is adversely affected. The unit decrease in purity of parental lines will eventually cause the severe yield loss in the F_1 hybrids. The quality of parental lines gets contaminated and deteriorated through mechanical mixtures during handling. Therefore, it is most indispensable to use pure nucleus and breeder seed of parental lines to use develop hybrids and nucleus and breeder seeds of A/B/R lines should be developed under the strict supervision of plant breeders as per below mentioned procedure:

NUCLEUS AND BREEDER SEED PRODUCTION OF A, B AND R LINES

Simultaneously, nucleus and breeder seed of A, B and R lines can be produced.

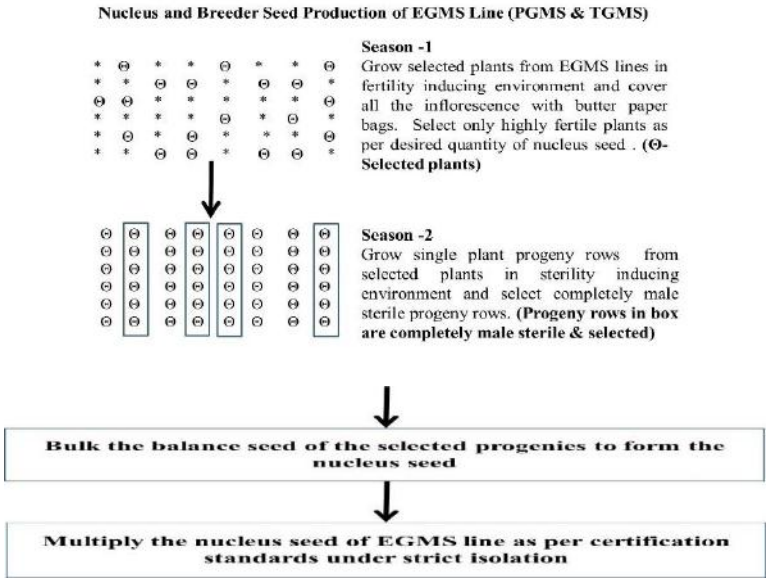


For A x B: Remove A x B crosses and their corresponding B line which lack uniformity in growth and flowering and show lack of stable male sterility. Rest of the B lines are bulked to produce nucleus seed of B line. Remaining, A x B crosses are used to produce nucleus seed of A line.

For A x R: Identify and remove the restorers which exhibit poor fertility restoration and less uniformity and rest of the Restorers are used to produce nucleus seed of R line.

NUCLEUS AND BREEDER SEED PRODUCTION OF EGMS (PGMS/TGMS) LINES

EGMS lines multiplied continuously for several generations without any selection may segregate for sterility and critical sterility problems which may deteriorate the quality and genetic purity. Therefore, nucleus and breeder seed production of EGMS lines has to be taken up on a continual basis and as per following procedure:



MAINTENANCE OF INBRED LINES

Inbred lines are generally used as parents in hybrid seed production through conventional method of hybrid development. Inbreds are developed from improved varietal population- (OPVs, composites and synthetics), Local varietal populations, Inter-varietal or interspecific hybrids and Gene pools. The following points have to be undertaken in consideration for maintaining the genetic purity of inbred lines:

- Inbred lines are maintained by both selfing and sib mating.
- Incompatible lines have to be maintained by sib mating.
- Uniformity for highly heritable characters is important for distinctness, stability and uniformity and it is Difficult to maintain the genetic purity and it needs personal attention also.

PROBLEMS ASSOCIATED WITH HYBRID SEED PRODUCTION:

- Existence and maintenance of the genetic purity of A, B & R Lines is laborious and difficult.
- In GMS based hybrid seed production plot identification of fertile line and rouging for them is laborious and required skilled labour and only yield will be up to 50 % only.
- If fertility restorer gene is not found for CMS line then use CMS system is not feasible in crops where seed is economical part.
- GMS is less stable than CMS and breakdown of male sterility is also observed under unfavourable environment.
- If exotic male sterile lines are not suitable to prevailing environment. Hence, the native/adaptive lines have to be converted into MS lines i.e., cumbersome or lengthy procedure.
- Adequate cross pollination should be ensured between A and R lines for good seed set.
- Synchronization of flowering
- Fertility restoration should be complete otherwise the F_1 seed will be sterile. Pollen shedders should not be there in the A line otherwise F_1 will not be true hybrid.
- Isolation is needed for maintenance of parental lines and for producing hybrid seed.
- Conversion of normal line to male sterile line or restorer line is time consuming and difficult process.
- EGMS system required two environment conditions to regulate fertility and sterility i.e. possible only countries where altitude and latitude variation is more.

HYBRID SEED PRODUCTION IN RICE

Origin of High yielding variety	: Dwarf gene of the mutant variety [Dee-Gee-Woo-Gen] (DGWG) discovered in Taiwan in 1960

First Report on Heterosis	: Jones of USA 1926, In India: Ramaiah (1933)
Hybrid rice	: During 1964 by Yuan Long Ping of China **(Father of hybrid rice)**
Male sterility source	: Wild abortive (WA)
Commercial hybrid seed production	: Cytoplasmic genetic male sterility system
Genes for fertility restoration in CGMS system	: *Rf1 and Rf2*

Seed Multiplication work at different Stages:

Breeder Seed : A (A x B), B, R lines are raised separately under isolation.
Foundation Seed : A (A x B) and R lines grown separately under isolation.
Certified Seed : A and R lines are crossed under isolation to get hybrid.
Hybrid Seed Production System: Three-line method or CGMS system. Two-line method or environmental genetic male sterility (EGMS) system that involve PGMS and TGMS was developed in China and low temperature hilly areas of Tamil Nadu.

COMMERCIAL HYBRID SEED PRODUCTION TECHNIQUES

Land requirement: Select fertile soil. No rice variety to be raised for past season, should have protected irrigation and drainage system with sufficient sunshine. Should not be any serious disease or any insect problem

Isolation:

Space isolation: Foundation seed: 200 m Certified seed: 100 m

Time isolation: 20 days either earlier or later for other varieties compared with MS line.

Barrier isolation: 30m of wood lot / tall crops plastic sheets of 2m height

Season: April, May, December and January. Favorable climatic conditions during flowering for higher seed set: Daily mean temperature 24 – 30^{o}C and seed set adversely affected if temperature is below 20^{o}C and above 35^{o}C. Relative Humidity 70 - 80 %. The difference between day and night temperatures should be 8-10^{o}C. Sufficient sunshine and moderate wind velocity of 2-3 m / second. Free from continuous rain for above 10 days during peak flowering season.

Seeds: Purchase the seeds of A, B and R lines from authenticated source with tag.

Seed rate: Female: 20 kg /ha, Male: 10 kg /ha

Seed Treatment: Dormancy breaking: Soak in 0.5% KNO_3 for 16 h., Bio fertilizers: *Azospirillum* @300 g/ 10 kg of seed, Pest protection: Slurry treatment with Bavistin / Thiram @2g /kg of seed.

Main field Transplanting: A line: 25 days, R line: 14, 18, 20 days

Spacing: Between A line - (15 x 15cm), Between A and R line - (20 cm), Between R line - (30 x 15cm)

Nursery Management: Keep irrigation channels separately for the parental lines, For Dec-Jan sowing take up staggered sowing for male twice or thrice with the interval of 10-15 days (3,10,15 days for effective seed setting), Keep the nursery area free of weeds. Apply DAP @ 2 kg / cent as basal to get vigorous seedlings. For April-May sowing sow the male 5 and 10 days after female line. Even split application of fertilizer N is favourable for production of vigorous seedlings.

Weeding: Pre-emergence herbicide Butachlor @ 2.5 litre / ha. Hand weeding is done before panicle initiation

Synchronization of flowering: Since mal sterile line (A line) depends on male parent (R line) for supply of pollens it is very important to see that they come to flowering simultaneously. It is better, if A line starts flowering early by 2 days because the stigma remains receptive for 4-5 days and total flowering duration of A line is 2-3 days more than male parent.

Irrigation: Field should have 5cm of standing water.

Supplementary Pollination: Application of 2% DAP spray to late parent. **Rope pulling** - moving of rope from male to female line in wind direction. **Rod driving** – moving rod from male to female row in wind direction. Leaf clipping (more than 2/3 of flag leaves are removed). GA_3 application @ 40-65 g/ha

GA_3 Spray:

- Application of GA_3 can adjust physical and biochemical metabolism of rice plant and helps in hybrid seed production by stimulating the elongation of young cells.
- In most of the CMS lines, about 20-30% of the spikelets of a panicle are inside the flag leaf sheath (exertion is only 70%).
- GA_3 effects exertion of panicle completely out of flag leaf sheath.
- The dose of 65 g/ha using knapsack sprayer and 30 g/ha with ultra-low volume sprayer is recommended.
- The application of GA_3 is recommended in 3 splits from panicle initiation days as follows: 1st Spray: At 10 % of the

panicle initiation. 2nd Spray: Next day of first spray. 3rd Spray: Next day of second spray.

- Spraying should be done at 8 to 10 am and 4 to 6 pm.

Advantages of GA_3 application: Enhances panicle and stigma exertion. Speed up growth of late tillers and increase effective tillers. Flag leaf angle is increased and reduces unfilled grains. Enhances seed setting and seed yield.

Rouging

Plants to be removed	A line	B line	R line
Diseased plants	All	All	All
Parental lines	R line & B line	A line & B line	R line & A line
Early flowering plant	All	All	All
Pollen shedders	All	-	-

Rogues / off types: Based on variation in phenotypic characters

Physiological Maturation

Duration: 27-30 days after flowering

Symptom: Straw yellowing of grain

Harvest: When 80% of the population, the seed become straw yellow in colour the crop is ready for harvest (Harvestable maturation). The male parent is harvested first. Care should be taken to avoid admixture of male and female line. Female line should be threshed separately in a well closed threshing floor. Seeds dried under sun / shade to 12% moisture content.

Storage: Use cloth bag or gunny bag for short term storage. Use 700gauge polyethylene bag for long term storage. Cool places improve storability. Stack bags up to 8 bags height for protection of seed quality avoiding crushing of lower bags.

Field inspections: Minimum four inspections shall be done as follows- **First inspection:** Before flowering in order to determine, isolation, presence of volunteer plants, outcrosses, planting ratio, errors in planting and other relevant factors. **Second and third inspection:** during flowering to check isolation, off types and pollen shedders in female parent. **Fourth inspection:** At maturity and prior to harvesting to verify true nature of the plant

Seed Yield: Hybrid yield (F_1): 1500- 2000 kg ha^{-1}

HYBRID SEED PRODUCTION IN SORGHUM

Male sterility system for Commercial production: Cytoplasmic genetic male sterility
Stages of seed multiplication: Breeder seed – foundation seed – certified seed.
Seeds produced in different stages

Nucleus seed stage: Maintenance of basic source by seed to row progenies.
Breeder Stage: A (AxB), B and R line are multiplied
Foundation Stage: A (AxB) and R line are multiplied
Breeder and foundation seed stage : Multiplication of male sterile line or maintenance of A and B line
Certified seed stage : A x R – F1 hybrid produced.
Certified seed stage : Production of hybrid seed
Foundation seed production : A and B line are raised in 4:2 ratio with 4 rows of B line as border row and allowed for cross pollination. The seeds from A line will be collected as A line seeds (multiplied).
Certified seed production : Hybrid seed production

COMMERCIAL HYBRID SEED PRODUCTION TECHNIQUES

Land requirement: Should be fertile with good drainage. Previous crop should not be sorghum. Avoid problem soils

Season: Best season November – December; Flowering coincide with rain will result in washout of pollen. Temperature for seed setting 37°C

Isolation distance	FS	CS
Normal	300	200
On presence of Johnson grass	400	400
On presence of forage sorghum	400	200

SEEDS AND SOWING

Seed: Must be from authenticated source. Use suitable class of seed (Foundation seed for certified seed production)

Seed rate: A line : 8 kg ha^{-1} R line : 4 kg ha^{-1}

Pre-sowing treatment: Seed hardening with 2% Potassium dihydrogen phosphate for 16 h with seed to solution ratio of 1:0:6 and drying back to original moisture content. Seed pelleting with pungam leaf powder @ 300g/kg of seed. Seed treatment with 5% carbofuran 3G to protect seed from shoot fly infection.

SOWING: Type of sowing - Either by direct sowing or transplanting; **Type of nursery -** Raised bed

Advantages of transplanting: Main field duration reduced by 10 days. Shoot fly attack at initial stage can be minimised. Seedling with chlorotic, downy mildew and attack may be eliminated. Population can be maintained. Seed rate reduced by $1/5^{th}$

Sowing depth: 2 cm

Field preparation: Ridges and furrows

Spacing: A line: 45 x 30cm, R line: 45 x solid row spacing.

Seed production field:

Field preparation: Ridges and furrows

Planting ratio: Foundation seed stage: 4:2(A:B) & Certified seed stage: 5: 2 (A:R)

Border rows: 4 rows of male (either B or R line) to, supply adequate pollen.

Live markers: Live plants used for identification of male line and It should have distinguishable morphological characters. Live markers can be sunflower, daincha etc.

Manures and Fertilizers: Compost: 12.5 t / ha; NPK: 100:50:50 kg ha^{-1}, Basal: 50:50:5 kg ha^{-1}, Top dressing: 25 kg N after last ploughing & 25kg N after boot leaf stage (45 DAS), Foliar spray: Spray 2% DAP thrice at 10 days interval after 1^{st} flowering to enhance seed set. For problematic soil: In calcareous soil spray 0.5% $FeSO_4$ thrice during crop growth (30, 40 & 50 days after sowing) to male plant to improve pollen viability and to enhance seed set.

Synchronization Techniques to increase seed set: Give hardening seed treatment to late parent and pelleting to early parent. Take up staggered sowing depending on hybrid and location. Application of 1% urea spray to lagging parent or primordial initiation stage (35-40 days). Withhold irrigation to the late parent to make early flowering. Spray malic hydrazide 500ppm or CCC 300 ppm to the advancing parent at 45^{th} day

Roguing: Do it in both parents. **In female line remove:** off types, wild types, pollen shedders, rogues, partials, volunteer plants, diseased plants, R line, mosaic plants, late / Early flowering plant. **In male line remove:** Rogues, A line, Diseased plants, Late / early flowering plants, Wild types

Weed Management: Spray atrazine 50WP @ 500 g ha^{-1} on 3^{rd} day after sowing as pre-emergence herbicide. Use sprayers fitted with flat nozzle using 900 litre of water per hectare. The field should be weed free up to 45 days. Hand weeding done of 30-35 days.

Irrigation: 1^{st} irrigation: Immediately after sowing; Life irrigation: 3^{rd} day after sowing; Subsequent irrigation: Once in a week. Critical stages: Primordial initiation stage, Vegetative stage and Milky stage

Designated disease: 1. Kernel smut 2. Head smut;

Sugary disease of sorghum: It is specific to hybrid, occur due to low seed set, Spray rogor 0.03% (or) Endosulfan 0.07% Pre harvest sanitation spray, Bavistin @ 10 g / 10 lit, to avoid black mould and ear head bug.

HARVESTING:

Physiological maturation (PM)

Duration	: 0-45 days after 50% flowering
Seed moisture at PM	: Around 30%
Visual symptom	: Formation of sunken layer on seeds
Seed moisture content at Harvestable maturity	: Around 20-25%
Harvesting technique	: Harvest male first and then female
Effect delay harvest	: Mould attack, amenable for field damage, yield and quality reduced

THRESHING

Seed moisture content: 15-18%

Techniques: Beating with pliable bamboo sticks or Mechanical threshers to avoid damage

Drying: Dry under sun to reduce the moisture content to 8%

Seed Treatment: Thiram @ 2 g kg^{-1} of seed halogen mixture @ 3 g kg^{-1} of seed

Seed storage: Storability: 2-3 years

Storage insect: *Sitophilus oryzae* Moisture previous container: Cloth bag (for short term storage) Moisture vapour proof storage - 700-gauge polybag (long term storage)

Seed yield: 3000 kg ha^{-1}

HYBRID SEED PRODUCTION IN PEARL MILLET

Breeding Technique for hybrid seed production: Cytoplasmic genetic male sterility system (CGMS)

History of pearl millet hybrid: The first report on CGMS line was made by Burton and his coworkers at Tifton Georgia USA. The line is Tift 23A.

COMMERCIAL HYBRID SEED PRODUCTION

Land Requirement: Select fertile land, avoid problematic soil and Previous crop should not be the same crop variety / after variety.

Isolation: Foundation seed - 1000 m and Certified seed - 200 m

Season: Irrigated: March – April, June - July January – February, Rainfed: October -November

Seeds: Must be from an authenticated source (SAU, NSC Department of Agriculture). Use proper stage for production (e.g., Foundation seed for certified seed)

Pre sowing seed Treatment: Treat with Metalaxyl @6 g kg^{-1} seed against downy mildew; Treat with *Azospirillum* 600g kg^{-1} seed for fixation of atmospheric nitrogen; Soak the seed in 10% NaCl solution to remove sclerotial bodies and ergot diseased seeds; Harden seeds with 2 % KH_2PO_4 for rainfed sowing.

Seed rate: A line - 6 kg ha^{-1}; B line - 2 kg ha^{-1}

Main field preparation: Ridges and furrows

Sowing: Seedling / hill : 1 seedling / hill

Planting ratio: Foundation Seed - 4 : 2 and Certified Seed - 6 : 2

Border rows: Foundation Seed - 8 (B line); Certified Seed - 4 (R line)

Depth of sowing: 2-3 cm

Spacing: A line - 45 x 20 cm; R line - 45 x solid row.

Nursery: Seedling can also be raised in raised bed nursery and can transplanted to the main field at 20-25 days of aging.

Manures & Fertilizers: Compost - 12.t ton/ha; NPK 100:50:50 kg ha^{-1} ; Basal: 50:50:50 kg ha^{-1} ; Top dressing: 50:0:0 kg ha^{-1} (At tillering phase)

Foliar spray: DAP 1% at peak flowering to enhance flowering and seed set.

Steps for synchronization of flowering: With holding irrigation; Application DAP 1%; Staggered sowing and Jerking

Jerking: It is done 20-25 days after transplanting or 30-40 days after direct sowing. The early formed ear heads of the first tillers are pulled out or removed which will result in uniform flowering of all the tillers.

Specialty with pearl millet in synchronization: The synchronization problem is less in pearl millet due to-Tillering habit; Supply of continuous pollen; Lesser pollen weight; Flight capacity of pollen; Pollen viability & stigma receptivity are longer.

Irrigation: 1st irrigation - Immediately after sowing; Life irrigation – 3rd day after sowing; Subsequent irrigation - Once in a week; Critical stages: Primordial initiation stage, Vegetative stage and Milky stage

Roguing: A line - seek for off types pollen shedders; R line -Seek for early flowering plants rouges and diseased plants.

Character of off types: Variation in leaf colour, leaf waviness, grain colour ear head, shape, size, etc.

No. of field inspections: Three i) Seedling stage ii) Tillering stage iii) Grain formation stage

Plant Protection: Aphids, Jassids - Monocrotophos, Rogor 2.5ml/lit; Ergot disease - Carbendazim @500 g/ac/ Mancozeb 1kg/ac (1st at 5-10% flowering & 2nd at 50% flowering); Downy mildew - Spray of Metalaxyl @ 500 g ha-1 / Ridomil WP @2 kg ha^{-1} / Mancozeb 1 kg ha-

Harvesting: Physiological Maturation - 30-35 days after 50 flowering; Visual symptoms - i) Seed colour changes from green to straw yellow in colour and ii) Formation of sunken layer at the point of attachment to the panicle; Moisture content - 30-35%

Harvesting Technique: Due to tillering habit, harvest the panicle / ear head in 2 picking and Select 5-7 tillers for seed purpose.

Threshing: Dry in yard for 2-3 days to bring moisture content to 15-18%; Stick beating (manual) or mechanical thresher (LCT Thresher).

Processing: Grade with 4/64" round perforated metal sieve as middle screen. Use OSAW cleaner cum grader

Seed Treatment: Thiram / Bavistin @3g kg-1 seed

Seed storage: Cloth bag for short term storage (12 months); 700-gauge polyethylene bag – long term storage (> 24 months)

Seed yield: 3200 - 3250 kg / ha

HYBRID SEED PRODUCTION IN MAIZE

System of Hybrid seed production: Datasseling or CGMS

Types of hybrids

Single cross hybrid Production: It is a cross between 2 genotypes A x B. A genotype will be de tasseled and crossed with B genotypes.

Double cross hybrid Production: It is a cross between 2 hybrids (A x B) x (C x D) (A x B) single cross hybrid will be produced by detasseling A and by crossing with B (C x D) hybrid will be produced by detasseling C and crossing with D. Then (A x B) will be detasselled and crossed with (C x D) hybrid.

Three-way cross hybrid Production: It is a cross between a hybrid and a variety or inbred. (A x B) x C (Inbred / genotypes). A x B) single cross hybrid will be produced by detasseling A and crossing with C. (A x B) progeny is detasseled and crossing with C.

Top cross: It is first generation resulting from the crossing of on inbred line and a certified open pollinated variety. (A x variety)

Double top crosses: The first generation resulting from the controlled crossing of a certified single cross and a certified open pollinated variety. (A x B) x variety

Hybrid seed production technique:

Land selection: Field should be free from volunteer plants, Well drainage system and Well fertile land

Field standards for isolation: For inbred lines (Foundation seed)

a) Same kernel colour : 400 m
b) Different kernel colour : 600 m
c) Some in bred not conforming to varietal purity : 400 m

For (foundation single crosses and hybrid of certified class)

	Foundation stage	Certified stage
Same kernal color	400	200
Different kernal colour	600	300
Field of single cross not confirming to varietal purity	400	200
Single cross with same male parent confirming to varietal purity	5	5
Single cross with other male parent not confirming to varietal purity	400	200

- Differential blooming dates are permitted for modifying isolation distance provided 5.0% or more of the plants in the seed parent do not have receptive silk when more than 0.20% of the plants in the adjacent field within the prescribed isolation distance are having shedding pollen.
- In hybrid seed production (certified seed stage) alone the isolation distance (less than 200 meter) can be modified by increasing the border rows of male parent, if the kernal colour and texture of the contaminant are the same as that of

the seed parent.

Seed production stages and production of parental lines / hybrids

Stage of seed	Single cross	Double cross	Three-way cross	Double top cross	Top cross
Breeder seed	A, B	A, B, C, D	A, B, C	A, B, variety	A, variety
Foundation seed	A, B	(AxB) (CxD)	(AxB), C	(AxB) variety	A, variety
Certified seed	A X B	(AxB) x (CxD)	(AxB)x C	(AxB) x variety	Ax variety

Seed production in maize hybrids: Land preparation - Ridges and furrows; Season - Second week of June to mid-July; Source of seed - Authenticated defined class of seed; Seed rate – Female - 7 kg ha^{-1}, Male - 3 kg ha^{-1}

Spacing: Female: 60 x 20 to 75 x 30 depending on the area and Male :45 x 30 cm

Depth of sowing: 5-6 cm

Planting ratio: Single cross: 4 : 2 Double Cross Hybrids : 6 : 2

Border rows: Can be modified based on isolation requirement. Minimum of 4 is best, Permanent structure can be used as border rows

Fertilizer: NPK kg / ha - 200 : 100 : 100; Basal - 100 : 100 : 50; 1st Top - 50 : 0 : 0 (20th days -vegetative phase); 2nd Top - 50 : 0 : 50 (Boot leaf stage at 45 days); Foliar - DAP 2% at 50% flowering. In Zn deficient soil - $ZnSO_4$ @ 25 kg ha^{-1}

Irrigation: First - On the date of sowing; Life - 3rd day; Regular - Once in 7-8 days; Critical stages – Boot leaf, tassel formation, floweringcob formation, silk emergence, milky and dough stage

Weed control: Pre-emergence herbicide - Atrazine @ 1 kg in 1000 lit/ha Hand weeding - 25 to 30 days after sowing; Caution - Do not enter into the field after boot leaf stage

Number of field inspections: Four (Seed certification officers) - One: Before flowering; Three - During flowering

Plant protection: Stem borer - Carbofuran / roger spray; Pink borer – Endosulfan; Aphids - Roger / Monocrotophos; Downy mildew - Metelaxyl, spray; Leaf rust / smut - Bavistin / Dithane spray; Root rot - Bavistin drench

Seed maturation: 14-20 DAA - milky stages (starch in fluid stage); 35 DAA - Soft dough stage; 45 DAA - Glazed dough stage; 55 DAA - Ripe dough stage

Symptom of Physiological maturation: The funicular degeneration; Formation of dunken layer; Moisture content of seed 35%; Cob sheath turn straw yellow colour;

Harvest: Harvest when the moisture content falls to 20-25 %; Harvest male first and remove from the field and then harvest female

Seed yield: 2.5 - 3.6 t/ha

Post-harvest operations

Cob sorting: Remove sheath and check for kernel colour, shank colour, diseased cobs, kernel arrangement etc.

Xenia: Effect on kernel colour or endosperm due to foreign pollen

Matezenia: Effect of a pollen on developing maternal tissue of a seed or fruit outside the embryo and endosperm due to foreign pollen

Shelling: Moisture content 15%, Mechanical (cob sheller), Manual (rubbing with stones)

Improper shelling leads to: 48% damage to kernels, Growth of storage fungal and Pericarp damage

Pericarp damage: Crack on pericarp and can be identified by $FeCl_3$ or Tz test

HYBRID SEED PRODUCTION IN PIGEON PEA

Technique for hybrid seed production: Genetic male sterility

Stages of seed production: Breeder seed – foundation seed – certified seed

Production particular with stage of seed

Breeder seed - Multiplication of female and male line in isolation

Foundation seed - Multiplication of female and male line in isolation

Certified seed - Production of F_1 hybrid

Control of male sterility - Monogenic recessive gene are maintained in heterozygous form following the principle of test cross.

No. of male sterility system reported: Two (MS_1 – Translucent white anthers and MS_2 - Dark brown, arrow head shaped anthers)

Hybrid seed production technique

Land requirement: Fertile land with an irrigation source. Previous crop should not be pigeon pea. Isolation distance is 200 m on all side from any other variety / hybrid of pigeon pea.

Fertilizer: Farmyard manure @ 20 cert loads ha^{-1}, N P K @ 25:50:25 kg ha^{-1}. DAP 25 kg as basal and 2% DAP spray at flowering and another after 15 days.

Seeds and Sowing: The female and male parents are sown simultaneously. Planting ratio: 4:2 (Female to Male) and If insect activities is more 6:2. Border rows- Two (around the plots). For hybrid seed production a ratio of 4:2 or 6:2 or 4:1 of male sterile pollen parent is to be adopted depending on honey bee activity. If bee activity is normal a ratio of 4:1 can be adopted. If honey activity is very less a ratio of 4:1 can be adopted. If honey activity is very less a ratio of 4: 2 may be adopted. If honey activity is moderate adopt a ratio of 6: 2.

Spacing: 60 x 20 cm

Sowing depth: 2-3 cm

Seed rate: Female parent: 40 kg ha^{-1}, Male parent: 5 kg ha^{-1}

Pre-sowing seed treatment: Rhizobium @ 3 pocket/ha or n—$ZnSO_4$ socking in 1/3rd volume (100 ppm)

Season of sowing: First fortnight of June or First fortnight of December

Supplementary pollination: To increase the activity of insects, the whole plot should be bordered with sunflower to increase bee activity to effect cross pollination. Bee hives may be placed @ 5. ha^{-1} for effective cross pollination.

Irrigation: First irrigation after sowing. Life irrigation on 3rd day. Subsequent irrigation depending on need once in 7-10 days. Mulching helps in moisture conservation

Rouging:

In male sterile line or female parent: Remove the off-type plant, Remove the male fertile line by examining the color of the anthers at the time of first flower formation, i .e. one day before flower opening. Roguing should be completed in7-10 days' time. Remove the late flowering plants.

In male fertile line or male line: Rogue out off-types. Remove the immature pods set in the plants from time to time to induce continuous flowering and to ensure pollen availability for longer time.

Weeding: Ensure weed free condition. Apply pre-emergence herbicide Basilin @ 1.5 litre /ha on 3rd day after sowing.

Harvesting - Physiological maturation 27.30 days. Symptom - Brown pods, tan colour of seed Collect the pods from the female parent which will be the hybrid seed.

Plant protection

Insects: Common problem blister beetle. Minimize insecticidal spray as it may kill the honey bees and other insects responsible for pollination and seed set. Spray NPV at 500 lit/ha with 20% teepol against pod fly. Spray endosulfan 4% or carbaryl 5% @ 25 kg or monocrotophos @ 625 ml/ha against pod borer. Spray neem oil 5% spray during flowering and pod set stage followed by Tricophos 0.05 % spraying.

Diseases: Sterility mosaic virus - Affected plant at young stage are removed. Spray monocrotophos @ 500 ml/ha as the symptoms are visible and continue with another spray after 15 days.

Wilt and root rot - Around the roots of all plants either affected or not, apply carbendazim @ 0.5 g dissolved in 1 litre of water.

Grading: Seed moisture content to be reduced to 16-14%. Use 10/64" round perforated sieve irrespective of parental and hybrid seeds. Reduce the final moisture content between 8-10% for prolonged storage.

Seed treatment: Treat seeds with Thiram/Bavistin @ 2g / kg^{-1} of seed along with carbaryl @ 200 mg kg^{-1} of seed. Treat the seed with halogen mixture @ 3g kg^{-1} of seed as ecofriendly treatment. Treat the seed with Turmeric rhizome power / chilli powder / neem leaf power @ 100 g kg^{-1} of seed for dual purpose seed storage.

Storage: Use cloth bag for short term storage. Use sealed container or 700gauge polythene bag for long term storage.

HYBRID SEED PRODUCTION IN SUNFLOWER

Technique for hybrid seed production: Cytoplasmic Genetic male sterility system

Commercial Hybrid seed production techniques:

Land selection: Select fertile & well drained soil; Avoid wilt / Charcoal rot infected field; The previous crop should not be sunflower past 2 seasons; Sunflower can tolerate high pH up to 8.5

Isolation: Isolate field from same variety or other varieties not confirming to certification stand all around the plot. The distance of foundation stage: 400m and certified seed stage: 200m

Land Preparation: Deep ploughing

Season: April – August, December – January. There should not be rain at the time of flowering.

Spacing: 45 x 30 cm (Female) and 45 x 30 cm or 45 cm line sowing (Male)

Fertilizer: N PK – 60 : 45 : 45 Kg ha^{-1}., FYM : 12.5 t / ha; Micronutrient deficiency: *Mn deficiency :* Basal 25 kg /ha (or) 0.5% $MnSO_4$ spray of 30, 40, 50 DAS; *Zn deficiency* : ZnSo4 Basal 25kg / ha (or) 0.5% $ZnSO_4$ spray at 30 , 40 & 50 DAS.

Seeds and sowing: Get seed form authenticated source. Get appropriate seed based on class of seed production (eg) Foundation seed - A & B line seeds and Certified seed - A and R line seeds. If dormant soak in 0.5% $KN0_3$ solution for 16 hrs. Treat with Thiram @ 2gKg^{-1} of seed.

Seed rate: A : 6 kg / ac and R : 4 kg / ac

Sowing depth: 2-3 cm

Planting Ratio: 3 :1 and Border row - 4

Herbicides: Apply Fluchloralin 2.0 l ha^{-1} before sowing or as pre-emergence spray.

Irrigation: At the time of sowing; Life irrigation (3^{rd} day); Once in 8 – 10 days.

Critical stages: i) Bud development ii) Seed development iii) Seed maturation

Rouging: Based on stem hairiness, leaf blade, leaf colour; Bract colour, find the off type and remove; Based on head shape; Convex, concave flower (disc floret colour, ray floret colour) off type are to be removed; Keep the florets upside down on around to avoid cross pollination by insects; Remove downy mildew effected plants

Supplementary pollination: Use muslin cloth and rub on male 1^{st} and then on female heads (morning hours 8.00 - 11.00 am). Keep bee hives @ 5-7 / ha

Special problem: Bird damage / parrot damage (Occur on milky stage seeds eaten away by birds) **a)** Bird scaring b) Coloured ribbon are blown.

Physiological maturation: Thalamus turns greenish yellow in colour.

Harvesting: Remove male first, then female; Moisture content: 15%; Do not heap the heads

Threshing: Dry and beat with sticks; Sunflower thresher (risky)

Grading: Sieve grading with - 8/64, 10/64" depending on parents of hybrids; Specific gravity grading is best.

Storage: Thiram treatment @ 2g kg^{-1} of seed; Seed moisture content: 8%; Cloth bag for short term storage; Polyethylene bag (700 gauge) at which 5-6 % seed moisture for long term storage

HYBRID SEED PRODUCTION IN CASTOR

Hybrid Seed Production Techniques: Use of 100% pistillate line. (Depending upon environment i.e. Temp. sensitive)

Land requirement: Select fairly deep, fertile and well-drained soil. Avoid alkalinity/salinity soils. (Problematic soil). Previous crop should not be castor. Fertilizer

ISOLATION

Isolation	Isolation distance (m)	Statutory isolation limits (m)
Male Parents		

Nucleus seed	1500	-
Foundation	1000	300
Certified	600	150
Female Parents		
Nucleus seed	2000	-
Foundation	1500	300
Certified seed of common hybrid	1000	150

Land Preparation: Deep planting, 2-3 harrowing

Stages of seed production: Breeder seed - foundation seed - certified seed

Area/regions	Western and Northern state	Southern state
Season		
Male Parent	July first Fortnight (FN)	
Female	July first FN	
Certified (Hybrid)	August season FN	Sep second FN
Spacing: Initial (cm) 90 x 30, Final spacing to be adjusted at the time of second ranging (cm) 90 x 60		

Seed rate (kg/ha): 10-12

Sowing: 4 to 5 cm deep

Row ratios: 4:2 or 3:1 (depending upon hybrid)

Nutrient Management: Fertilizer - N P K kg/ha 80: 60: 0; Basal - 40: 60: 0; After 45 to 60 days - 20: 0: 0; After first picking - 20: 0: 0

Herbicides: Plot should be weed free during first 45 days of crop growth. Spray Fluchloralin or Trifluralin @ 1 kg active ingredient / ha. 3-5 days prior to seedling.

Irrigation: Depending upon the soil and the crop season. Kharif - 4-6; Rabi - 6-8; Summer - 15-20 at an interval of 9-10 days.

Plant Protection: Caster semilooper - Monocrotophos (0.05%) or quinolphos (0.05%) or dimethoate (0.05%) or endosulfan (0.05%) 10-15 days. Tobacco caterpillar - Chlorphyriphos (0.05%); Caster hairy cater pillar - Phosphomidon or quinolphos (0.05%) MCC or twice at 10 days interval.

Sex expression: Occurrence of staminate flower mostly related to seasonal variation and associated with the genotype and mean day temperature. Generally female tendency is highest in rabi and early summer. Plants tends to be mostly made when planted. In late summer and kharif. Temp below 32°C: Mostly female. above 32°C - Plant produces more male flowers. Besides temp. age of plant and level of nutrition and influence sex expression. Female tendency is in general highest in young plant with high level of nutrition. White reverse in the case with old and poorly nourished plants.

	Roguing	:	Minimum 3 field inspection requires.
	Crop growth stage		**Basis for identification**
i)	At least 10 days prior to flowering in primary raceme	:	Stem colour, internode type, leaf shape and bloom.
ii)	Flower initiation primary raceme in	:	Nodes up to primary raceme, internode type, sex expression, branching and spike characters.
iii)	Flower initiation secondary order raceme in	:	In female parent spike and capsules character in primary raceme and reversion to monoecious in secondary order.
iv)	Flower initiation Ternary order raceme in	:	In female parent Reversion to monoecism in tertiary and quaternary order racemes.

Physiological Maturation: When capsules turn green to pate yellow– brown colour or 1 or 2 capsules dried.

Harvesting: First harvest female line (hybrid) capsules harvested sequenced order racemes. Generally, two picking required starting from 90 to 120 days at an interval of 25-30 days.

Threshing: After harvesting capsules dried in sun for 3–7 days. Seeds may be separated from capsules either manually or mechanically. Keep picking seed lots separately.

Grading: Sieve grading with 18/64". Depending upon genotypes or hybrid.

Storage: Seed moisture polyethylene bag content 8.00 cloth bag

HYBRID SEED PRODUCTION IN COTTON

Hybrid Seed Production Techniques: Manual method / Emasculation and dusting.

Other breeding systems for hybrid seed production: Genetic male sterility (eq. Suguna) Cytoplasmic genetic male sterility.

Commercial Hybrid Seed Production technique

Land Selection: Free of volunteer plants of cotton variety deep, well drained and fertile soil.

Land preparation: Fine tilth with giving ploughing followed by 2-3 harrowing.

Isolation: FS - 50m; CS - 30m; Between parental lines - 5m

Seed rate: Delinted: Female - 1.5 kg/ha; Male - 0.50 kg/ha;

Fuzzy: Female - 2.00 kg/ha; Male - 0.75 kg/ha

Spacing: Female - 120 x 90 cm; Male - 90 x 60 cm

Manures & Fertilizers: FYM - 25 tonnes/ha; NPK - 160 : 75 : 30 kg/ha; I dose at sowing - 50: 75: 30; II dose at 30 DAS - 30 : 0: 0; III dose at 50 DAS at square formation - 35: 0 : 0; IV dose at 60 DAS at flowering - 45 : 0 : 0

Sowing: Female & male parents are sown separately side by side in the ratio 4:1 or 5:1. (Adopting block system)

Pre-sowing seed treatment: Thiram or Captan @2 .5 g/kg of seed.

Irrigation Stages: No. of irrigation - 9

Critical stages: germination, square period, crossing period, boll formation period, boll maturity period etc. The frequency of irrigation depends on soil type, weather factors like rainfall, temperature, wind velocity etc. Irrigate the field once in 10-15 days depending upon soil type and weather conditions.

Roguing: From flowering initiation and continued till flowering is completed.

Characters for rouging: Leaf colour, shape, leaf hairiness, flower colour, petal eyespot, boll shape.

Picking: 30 to 40 percent boll bursting and Generally 3 to 4 pickings are required.

Seed yield: Kapas yield 15-20 q/ha and Cotton seed yield 7-10 q/ha

Delinting Methods

Acid delinting: Used concentrated H_2SO_4 (93 to 98%) @100 ml/kg of fuzzy seed for 3-4 minutes.
Dry gas delinting: Dry HCl gas is injected in a revising drum containing fuzzy seed. The drum is heated. Temp. reaches 49°C Ammonia gas is used for neutralize the acid traces.

Seed storage: Seed is dried up to 8-9% moisture and stored in well dried cloth bag.

HYBRID SEED PRODUCTION IN OKRA

Commercial hybrid seed production technique

System involved: Manual manipulation of sterility by emasculation & dusting

Land requirement: Free from volunteer plants; Free from *Macrophomina* infection

Isolation: Foundation seed - 400m; Certified seed - 200m

Season: June-July; Sep-Oct

Spacing: 45 x 30 cm

Fertilizer: NPK @ 80 : 60 : 60 kg ha^{-1}

Seed rate: Female – 8 kg/ha, Male- 4 kg/ha

Planting method: Block system

Planting ratio: 8:1

Hybridization: Emasculation of female and dusting with male pollen

Distance between male and female: 5m

Roguing: From Vegetative to Harvesting stage

Field inspections: 3 i) 1st Before flowering ii) 2nd During peak flowering iii) 3rd Fruiting stage (matured fruit stage)

Physiological maturation: 30 days after anthesis

Harvesting: Picking 4-5

Problems in Harvesting: Shattering

Seed Grading: 10/6" round perforated Metal sieve; IDS method (Incubation drying & separation)

Seed Treatment: Bavistin @ 2g kg^{-1} of seed

Seed Storage: Long-term storage (HDPE/700 sieve poly bags)

HYBRID SEED PRODUCTION IN TOMATO

Hybrid seed production technique: Emasculation technique

Land and climate selection: Dry season 21-30º C/ 15-20ºC temperature, Poor fruit set at > 30^{O}C& >60% LH, Soil pH – 6.0 to 7.0, Low pH (15.5)

Season: January-February, Oct-Nov.

Selection of Parents and sowing: Female-best seed yielder.

Planting ratio: 1: 3 (Male: Female) adopting in block system, can be extended up to 4-5

Sowing: Staggering - Male parent sown 3 week earlier; Seed rate - Female -60-100 g/ha Male - 20-25g/ha

Nursery: Raised Bed.

Stages of seed production: Breeders, foundation seed, certified seed Parental line multiplication, (BS & FS) hybrid production (CS).

Isolation: FS : 200m, CS : 100m; Between parental line : 5m

Transplanting: 20-25 days old plant

Spacing: Female - 50 m Male - 40 cm

Stacking: Female –both for indeterminate & determine; Easy emasculation; Prevents rotting of fruits; Male –only for indeterminate

Hybridization technique

Emasculation: 55-65 days after sowing [Removal of stamens from flower buds of female line before they shed pollen; Select flower buds from second cluster which will open in next 2-3 days; Petals – slightly out of flower bud, but not opened; Corolla colour is slightly yellow or pale; Sterilize the forceps, scissors and gloves with 95% alcohol; Open the selected buds: split open the anther cone and remove; Calyx, corolla& pistil – intact; Cut few sepals; Preferably in the morning hours].

Pollen collection: Collect flowers from the male parent to extract pollen. Collect pollen early morning before pollen shed. Avoid pollen collection on rainy days. Remove anther cones & put in glassine envelopes. Dry under a 100w lamp for 24 hrs. (30^{O}C). Place the anther cones in a cup – cover with 200-300 mesh screen cover with lid. Fresh pollen – good seed set. Store pollen in sealed container under freeze & refrigerated condition.

Pollination: 1-2 days after emasculation [Corolla turns bright yellow; Dip the stigma into a pool of pollen; Continue for 3 –5 weeks; Remove non-crossed flowers /fruits].

Rouging: On the basis of plant type, leaf type, fruit characters (shape, size, color); Diseased plants yellow mottling, curling, cupping; Stunted plants.

Designated / seed borne diseases: Early blight (*Alternaria solani)*; Leaf spot; Tobacco mosaic virus (TMV)

Harvesting: Ripening 50-60 days after pollination; Be sure to check for clipped sepal; Use nylon net bags, plastic containers.

Seed extraction:

Manual: Harvest the fruit in nylon bags. Crush the fruit by trampling with feet. Put into plastic containers for fermentation. For one day – if Temp is > 28^{O}C for 2-3 days – if Temp < 28^{O}C. Fill the container with water and stir well. Remove the debris and wash the seeds.

Mechanical: Extract pulp – for crushing and separation of seeds and gel from pulp. Treat the seed gel mass with 7-10 ml HCl per kg of seed gel. Stir continuously for 40min/until the gel is dissolved fully. Wash thoroughly.

Seed drying: Place the washed seeds in fine mesh bags; Spin drying – for quick drying. Spread the seeds uniformly. Loosen the clumps of seed by hand. Dry the seeds in seed drier for 3- 4 days at 28 –30^{O}C. (6-8% M.C).

Seed packing and storage: Grading – 12 x12 BSS or 5/64 " size. Halogen mixture @ 3g/kg of seed; Store in vapour proof /air tight containers (4- 5yrs). Storage temp 20˚ C & 30% RH.

SEED SAMPLING FOR PHYSICAL PURITY ANALYSIS

Introduction

Seed lot: It is a uniformly blended quantity of seed either in bag or in bulk.

Seed size	Maximum quantity per lot
Larger than wheat and paddy	20,000 kg
Smaller than wheat and paddy	10,000 kg
Maize	40,000 kg

Sampling intensity

For seed lots in bags (or container of similar capacity that are uniform in size)

Up to 5 containers	Sample each container but never < 5 P. S
6-30 containers	Sample at least one in every 3 containers but never < than 5 P.S.
31-400 containers	Sample at least one in every 5 containers but never < than 10 P.S.
401 or more containers	Sample at least one in every 7 containers but never < than 80 P.S.

When the seed is in small containers such as tins, cartons or packets a 100 kg weight is taken as the basic unit and small containers are combined to form sampling units not exceeding this weight e.g., 20 containers of 5 kg each. For sampling purpose each unit is regarded as one container.

For seeds in bulk

Up to 500 kgs	At least 5 P.S
501 to 3000	I.P.s for each 300 kg but not less than 5 P.S
3001-20,000	I.Ps for each 500kg but not less than 10 P.s
20,001 and above	I.P s for each 700 kg but not less than 40.

PRINCIPLES OF SAMPLING: Sample is obtained from seed lot by taking small portion at random from different places and combining them. From this sample smaller samples are obtained by one or more stages. In each and every stage thorough mixing and dividing is necessary.

METHODS OF SAMPLING

Hand sampling: This is followed for sampling the non-free flowing seeds or chaffy and fuzzy seeds such as cotton, tomato, grass seeds etc., In this method it is very difficult to take samples from the deeper layers of bag. To overcome this, bags are emptied completely or partly and then seed samples are taken. While removing the samples from the containers, care should be taken to close the fingers tightly so that no seeds escape.

Sampling with triers: By using appropriate triers' samples can be taken from bags or from bulk.

Bin samplers: Used for drawing samples from the lots stored in the bins.

Nobbe trier: This name was given after Fredrick Nobbe, father of seed testing. This trier is made in different dimensions to suit various kinds of seeds. It has a pointed tube long enough to reach the centre of the bag with an oval slot near the pointed end. The length is very small. This is suitable for sampling seeds in bag not in bulk.

Sleeve type triers or stick triers: It is the most commonly used trier for sampling. There are two types viz., With compartments, and Without compartments.

It consists of a hallow brass tube inside with a closely fitting outer sleeve or jacket which has a solid pointed end. Both the inner tube as well as the outer tube have been provided with openings or slots on their walls. When the inner tube is turned the slots in the tube and the sleeve are in line. The inner tube may or may not have partitions.

This trier may be used horizontally or vertically. This is diagonally inserted at an angle of 30°C in the closed position till it reaches the centre of the bag. Then the slots are opened by giving a half turn in clock wise direction and gently agitated with inward push & jerk, so that the seeds will fill each compartment through the openings from different layers of the bag, then it is again closed and withdrawn and emptied in a plastic bucket. This trier is used for drawing seed samples from the seed lots packed in bags or in containers.

TYPES OF SAMPLES

Primary sample: Each probe or handful of samples taken either in bag or in bulk is called primary sample.

Composite sample: All the primary samples drawn are combined together in suitable container to form a composite sample.

Submitted sample: When the composite sample is properly reduced to the required size that to be submitted to the seed-testing lab, it is called submitted sample. Submitted sample of requisite weight or more is obtained by repeated halving or by abstracting and subsequently combining small random portions.

Working sample: It is the reduced sample of the required weight obtained from the submitted sample on which the quality tests are conducted in seed testing lab.

Weight of submitted sample: The minimum weight for submitted samples for various tests are as follows;

Moisture test: 100 grams for those species that have to be ground and 50 grams for all other species.

For verification of species and cultivar

Crop	Lab only (g)	Field plot & lab (g)
Peas, beans, maize, soybean and crop seeds of similar size	1000	2000
Barley, oats, wheat and crop seeds of similar size	500	1000
Beetroot and seeds of similar size	200	500
All other genera	100	200

For other tests like purity and count of other species

Crop	Size of seed lot (kg)	Size of submitted sample (g)	Size of working sample for purity (g)	Size of working sample for count of other species (g)
Paddy	25000	400	40	400
Wheat	25000	1000	120	1000
Maize	40000	1000	900	1000
Sorghum	10000	900	90	900
Bajra	10000	150	15	150
Red gram	20000	1000	300	1000
Green gram	20000	1000	120	1000
Black gram	20000	1000	150	1000
Bengal gram	20000	1000	1000	1000
Cowpea	20000	1000	400	1000
Soybean	20000	1000	500	1000
Groundnut (pods)	20000	1000	1000	1000
Groundnut (kernels)	20000	1000	600	1000
Gingelly	10000	70	7	70
Sunflower (varieties)	20000	1000	250	1000
Sunflower (hybrids)	20000	1000	125	250
Cotton linted (varieties)	20000	1000	350	1000
Cotton-delinted (varieties)	20000	350	35	350
Cotton linted (hybrids)	20000	350	35	350
Cotton -delinted (hybrids)	20000	250	25	250
Brinjal	10000	150	15	150
Chilies	10000	150	15	150
Bhindi	20000	1000	140	1000
Tomato - varieties	10000	70	7	70

Tomato hybrids	10000	7	7	7
Cabbage	10000	100	10	100
Cauliflower	10000	100	10	100
Knol-Khol	10000	100	10	100

The samples taken may be packed in bags, sealed and marked for identification. For moisture testing the samples should be packed separately in moisture proof polythene bag and kept in the container along with submitted samples.

INFORMATION TO ACCOMPANY THE SAMPLE

Date:
Kind Variety
Class of seed Lot No.
Quantity of seed in lot (kg):
Test(s) required: (1) Purity (2) Germination and (3) Moisture
Sender's name and address:

Types of sample used in seed testing lab

Service sample : Sample received from the farmers.
Certified sample : Sample received from certification agencies or officers.
Official sample : Sample received from the seed inspectors.

POST HARVEST HANDLING OF SEED TO MAINTAIN QUALITY OF SEED

A. Harvesting of seed

Advantages on correct method of harvesting: Seed yield will be protected without loss due to shattering. Processing loss will be reduced. Seed storability will be more.

Physiological maturation: The correct stage of harvesting for seed crops is termed as physiological maturation. It can be represented both as duration and visible symptoms.

Harvestable maturation: This is for the population. This will be later to physiological maturation. At this stage, more than 80% of the population will attain physiological maturation hence without economic loss crop can be harvested.

Caution on harvesting seed crop: Harvesting should be done after PM at HM.

Method of harvesting: Harvesting of crop can be done either mechanically or manually. Mechanical harvesting can be done only as single or once over harvest. Manual harvesting is done in two methods which is single harvest (or) periodical harvest.

Specialty with hybrid seed production in harvesting: Male should be harvested first and to be removed from the field before the harvest of female parent. Female should be harvested separately and brought to separate Threshing floor.

B. Grading and upgrading of seeds

Seed grading: It is done after threshing and before seed treatment in any seed production cycle. Grading is homogenation of a seed lot based on any one of the morphological characters of seed. Morphological characters used for grading are size, weight, colour, shape and surface texture.

Size grading: The seeds are initially graded based on size to bring uniformity in seed lot. It is also termed as basic grading. For size grading different sieves of uniform role size are used. For size grading the seeds, two different systems are used *viz.,* American System (AST) and British System (BSS).

Grading based on weight: Based on weight also seeds are being graded. It can be done either using water (Based the efficiency of buoyancy of seed to float due to the difference in seed weight). Using machine known as specific gravity separator. e.g., Paddy - upgrading technology; Marigold, Casuarina, Mild organic solvents used for specific gravity grading e.g. Acetone.

Grading based on colour: It can be done either manually or mechanically. The machine used for colour grading is electronic colour sorter.

Grading based on shape: Based on seed shape it can be graded. Seed shape vary as oblong, rectangular, round, triangular, square, hexagonal.

Grading based on surface texture: In processing magnetic separator is used for separating lucerne seed from dodder seed with corrugated surface texture.

Upgrading of seeds: Upgrading additional grading for further seed quality improvement.

Some of the machineries used in processing unit:

- ❖ Specific gravity separator - For grading seeds based on weight
- ❖ Indented cylinder - For grading seeds based on shape
- ❖ Electronic colour sorter - For separation of seed based on colour
- ❖ Magnetic separator - For separation seeds based on surface texture

Other machines based on specificity:

Sl. No.	Machine	Usage	Crop
1	Pod grader	Grading based on size	Groundnut
2	Ginner	Removal of fuzz form seed	Cotton
3	Delinting	Removal of lint from seed	Cotton
4	Tomato pulper	Pulping of fruits	Tomato

Grading of seed in relation to hybrid seed production: Normally grading sieve size will not be varying with parental lines / hybrid. If it varies each genotype should be graded with the specified sieves recommended.

C. Packing material for seeds: Packing materials classified into 3 based on their relation with moisture.

1. Moisture pervious container.
2. Moisture resistant container
3. Moisture vapour proof containers.

What is moisture transmission: When a seed is kept in an atmosphere, since both are having moisture, the transmission of moisture from one to another will happen till they attain uniformity in moisture which is known as moisture equilibrium status.

For long term storage seeds are to be stored in moisture vapour proof containers. Short term storage moisture previous containers are used.

Packing material for hybrid seed: Seeds of parental lines of hybrid are highly costlier. Hence it is preferable to pack in moisture vapour proof containers and keep them under a cool condition (> 5^0C).

D. Storability of hybrid seed: The storability of hybrid seeds normally will vary with parental lines. The female parents are usually poor storers than the male parent. The male parents are good storers while the maintainer lines are medium in storability. Hence the caution should be given to store the female line in seed storage conditions.

GENETIC PURITY TESTING

What is genetic purity?

Genetic purity (true to type or genuine): A count is made of the number of seeds, seedlings or plants that are true to this type. (degree of trueness or genuineness)

Need for genetic purity testing: To increase crop production at national level. To increase farmers income and standard of living. To make IPR (plant breeders right and plant variety protection) part strong. For distinctiveness, uniformity and stability (DUS) test. Quality control of grains for processing. Documentation of genetic resources.

Causes of deterioration of genetic purity: Mechanical mixture. Premature or unofficial release of variety. Improper certification. Genetic variation. Unstable seed parent

Methods of Genetic Purity Testing: 1. Morphological Testing 2. Chemical Tests 3. Molecular Marker Tests

Morphological Methods: 1. Seed morphology 2. Examination of seedlings 3. Examination in green houses 4. Grow out test

Seed morphology testing

- Characters like size and shape of grain, base of lemma, vertical crease hairs, rachilla hairs, deviation of lateral dorsal nerves wrinkling of lemma and palea etc.
- Morphological characters are examined with the aid of suitable magnification.
- The colour characteristics examined under full day light or light of limited spectrum e.g. ultraviolet light.
- Scanning electron microscope for studying differences in seed coat surface and its inner structure have also been used in some species.

Grow out test (GOT)

- Characters - Highly heritability; Stable expression over a range of environments; Easily discerned by visual observation.
- Sufficient spacing between rows and plants.
- Various samples of the same cultivar sown in succession and standard samples are sown at suitable interval
- Deviation from control sample counted
- Mutual comparison between the samples to be tested and the standard.
- Observations at full growing period

Mechanical vision: Acquisition of data using a video or similar system. Subsequently analyzing these data with the help of computer.

Image analysis: Extraction of numerical data from an acquired image. Shape descriptors used, because they are largely independent of size of the seed and so minimize the effect of environment and another factor.

Limitations of morphological methods: Environmental stress conditions often mask specific morphological traits. Large amount of land required. Laborious. Time consuming. Unfavorable condition, i.e. disease and insect infestation may limit GOT in field. Morphological markers are becoming limited in relation to rapid increase in number of varieties, hybrids and transgenics.

Chemical Tests: The test ranges from simple colour tests to complex chromatographic separations of phenols, anthocyanin, flavonoids and other compounds.

1. Phenol test
2. Peroxidase test
3. Fluorescence test
4. Modified phenol test
5. Potassium hydroxide –bleach test
6. Ferrous sulphate test
7. NaOH test
8. HCl test
9. Seedling pigmentation

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.

Biochemical Tests

Electrophoresis: Polyacryamide gel electrophoresis (PAGE) SDS-PAGE Isoelectric focusing (IEF) Ultra thin layer isoelectric focusing (UTLIEF)

General methodology for electrophoresis-based bio-chemical method

- Selection of plant material.
- Isolation of protein or isozymes.
- Electrophoresis.
- Staining of gel with different staining agents.
- Soluble protein 0.1% amido schwarz in 7% acetic acid.
- Esterase fast blue RR salt-alpha-naphthyl acetate.
- Catalase 0.1% potassium ferry cyanide in presence of 0.03% H_2O_2.

Advantages and limitations of Biochemical methods

- They are not affected by the field or greenhouse environment.
- They are cost effective compared to other methods and the turnaround time is relatively rapid.
- Multilocus analysis provide useful information for verifying inbred and hybrid genotypes.
- Most are co-dominant and many loci express at all stages of life cycle.
- An array of enzymatic analysis can be made using small quantities of leaf and seed material.
- There are limited number of marker isozymes as compared to molecular markers.

Molecular Markers Test

- RAPD (Random Amplification of Polymorphic DNA)
- SCAR (Sequence Characterized Amplified Region)
- SSR (Simple Sequence Repeats)
- STS (Sequence Tagged Site)

General methodology for molecular markers

- DNA extraction
- PCR amplification using nucleotide primer
  Initial Denaturation: Repeated Cycles, Denaturation, Annealing, Extension and Final Extension
- Electrophoretic run and identification of PCR amplified product.

Advantages and limitations of molecular techniques

- It has very large number of polymorphism development as compared to the bio-chemical markers.
- Residual heterozygosity can be detected.
- It is reliable to all crops.
- Very fast method.
- Sophisticated instruments required.

SEED STORAGE TECHNIQUES

Seed storage: It is the maintenance of high seed germination and vigour from harvest until planting.

Importance of seed storage: Seed storage is important to get adequate plant stands in addition to healthy and vigourous plants.

Factors affecting seed longevity in storage

I. **Genetic factors:** The storage is influenced by the kind/ variety of seeds. Some kinds are naturally short lived (e.g.) onion soybeans, ground nut etc., Within a crop the storage period varies between varieties. Also, the storage periods of hybrid and parent are differing.

II. **Pre harvest factors**
   a. **Effect of provenance:** (e.g.) Red clover seeds grown in Canada stored for 4 years with 80% germination whereas seeds grown in England and New Zealand stored for 3 years with 80% germination. This is due to different climatic conditions and soil types prevailing in different places.
   b. **Effects of weather:** Fluctuating temperature during seed formation and maturity will affect seed storage pre harvest rain may also affect the viability.
   c. **Pre harvest sanitation spray:** In pulses, insect infestation comes from field (e.g.) bruchids.

III. **Seed structures:** The presence or absence of glumes (lemma and palea) in grasses influence the storage period. Husk, chaff or both have shown an inhibitory effect on the growth of mould and an increase in life span of cereals seeds.

   Generally small seeds escape injury, whereas large seeds are more likely to be extensively damaged (e.g) bean, lima-bean and soybean.

IV. **Initial quality of the seed:** Seed lots having vigourous, undeteriorated seeds store longer than deteriorated lots.

V. **Environnemental factor**

a. Moisture content : The amount of moisture in the seeds is the most important factor influencing seed viability during storage.

Generally, if the seed moisture content increases the storage life decreases. If seeds are kept at high moisture content the losses could be very rapid due to mould growth very low moisture content below 4% may also damage seeds due to extreme desiccation or cause hard-seediness in some crops.

Since the life of a seed largely revolves around its moisture content it is necessary to dry seeds to safe moisture contents. The safe moisture contents however depend upon storage length type of storage structure, kind / variety of seed, type of packing material used. For cereals in ordinary storage conditions for 12-18 months, seed drying upto 10% moisture content appears quite satisfactory. However, for storage in sealed containers, drying up to 5-8% moisture content depending upon particular kind may be necessary.

b. Relative humidity and temperature during storage: Relative humidity is the amount of H_2O present in the air at a given temperature in proportion to its maximum water holding capacity. Relative humidity and temperature are the most important factors determining the storage life of seeds. Seeds attain a specific and characteristic moisture content when subjected to given levels of atmospheric humidifies. This characteristic moisture content is called equilibrium moisture content. Equilibrium moisture content for a particular kind of seed at a given relative humidity tends to increase as temperature decreases.

Thus, the maintenance of seed moisture content during storage is a function of relative humidity and to a lesser extent of temperature. At equilibrium moisture content there is no net gain or loss in seed moisture content.

c. Temperature: Temperature also plays an important role in life of seed. Insects and moulds increase as temperature increases. The higher moisture content of the seeds the more they are adversely affected by temperature. Decreasing temperature and seed moisture is an effective means of maintaining seed quality in storage.

The following are thumb rules by Harrington are useful measures for assessing the effect of moisture and temperature on seed storage. These rules are as follows:

a. For every decrease of 1% seed m.c. the life of the seed doubles. This rule is applicable between m.c. of 5-14%.
b. For every decrease of 5^{o}C in storage temperature the life of the seed doubles. This rule applies between 0^{o}C to 50^{o}C.
c. Good storage is achieved when the % of relative humidity in storage environment and the storage temperature in degrees Fahrenheit add up to one hundred but the contribution from temperature should not exceed 50^{o}F.

Nomograph: Roberts (172) developed formulae to describe the relationship between temperature, seed moisture content and period of viability. From these relationships it was possible to construct a seed viability nomograph. These nomographs are helpful in predicting the retention of seed viability in defined storage environment for a particular period or to determine combination of temperature and moisture content which will ensure the retention of a desired level of seed viability for a specific period.

d. Gas during storage: Increase in O_2 pressure decreases the period of viability. N_2 and CO_2 atmosphere will increase the storage life of seeds.

e. Microflora, insects and mites: The activity of all these organisms can lead to damage resulting in loss of viability. The microflora activity is controlled by R.H, temperature and m.c. of seed.

VI. Seed treatment: Treated seeds with fungicides can be stored for longer periods. Fumigation to control insects will also help in longer period of storage.

Fumigation: Once the seed storage is free of completely free of insects, the most serious source of reinfestation is infested seed, which is brought in. Seed may be brought from the field already infested, or it may be transferred from infested storage. Such infestation is controlled by fumigation. Fumigation is effective only in gas-tight storage. Numerous effective fumigants are available.

	Dosage	**Exposure period**
Methyl bromide	16 to 32 mg / cubic meter	24 hours
Hydrogen cyanide	32 to 64 mg/cubic meter	24 hours
Hydrogen phosphide	5 to 10 tablets per ton of seed	3 to 7 hours.

It must be borne in mind that fumigation, particularly repeated fumigation, may seriously reduce the vigour and even the germination capacity of seeds. This is particularly true of seeds with a high moisture content. Seeds with moisture content greater than 14 per cent should be dried to below this value before fumigation.

VII. Types of packing materials: Moisture vapour proof containers can help in longer storage than the moisture pervious containers.

VIII. **Use of desiccants:** Desiccant like silica gel can maintain the m.c. in equilibrium with the R.H. of 45%. It is kept @ 1 kg/10 kg of seeds. When the blue silica gel turns to pink colour it should be dried at 175^{o}C in oven and then again placed in the container.

SEED PACKING MATERIALS

Seed packing: Is the process of filling, weighing and sewing of bags with seed. Factors to be considered while selecting the packaging materials are: Kind of seeds to be packed; Quantity of seed; Value of seed; Cost of packaging material; Storage environment in which the packed materials will be held; Period of storage.

Classification of packaging materials or containers

1. **Moisture and vapour pervious containers:** These containers allow entry of water in the form of vapour and liquid. These are suited for short-term storage. The seeds in these containers will attain seed equilibrium moisture with the surrounding atmosphere (e.g.) cloth bags, gunny bags, paper bags etc.,
2. **Moisture impervious but vapour pervious containers:** These allow entry of water in the form of vapour and not in liquid. The seeds in these containers can't be carried over for long period in hot humid conditions. (e.g.) polythene bags of > 100-gauge thickness and urea bags.
3. **Moisture and vapour proof containers:** These containers will not allow entry of moisture in the form of liquid or vapour. These are used for long term storage even in hot humid conditions if the seeds are sealed at optimum m.c (e.g.) polyethylene bags of >700-gauge thickness, aluminum foil pouches, rigid plastics etc., Certified seeds of cereals, pulses and oil seeds are normally packed either in gunny bags or cloth bags. However, paper bag, aluminum foil pouches and polyethylene bags are used for packaging flower and vegetable seeds.

Types of seed storage based on category of seed: The type of storages are based on the time of storage. It can be classified into 4 types.

a. **Commercial seeds:** The largest storage need is for the storage of seed from harvest until planting. The storage period ranged from a few days to 8 or 9 months. Here seeds must be dried to a m.c of < 14% for starchy seeds and less than 11% for oil seeds.
b. **Carryover seeds:** About 20-25% of stored seeds may have to be carried over through one growing season to the second season. This storage period is usually between 1-year &11/2 years. Seeds can be stored in steel bins with tight fitting lids or in moisture proof bags.
c. **Foundation seed stocks:** F.S can be stored for several years, since genetic drift is minimized by reproducing foundation or stock seeds. This seed can be stored at R.H. of about 25% and temp. at 30^{o}C or a R.H. of 45% and temp. of 20^{o}C. This can be achieved by using a dehumidifier. Store the seeds with polythene bags of > 700-gauge thickness.
d. **Germplasm seeds:** These seeds are to be stored for many years. Basic requirements for such very long-term storage are coldest temperature and seed m.c. in equilibrium with20-25% R.H. storage rooms can be, maintained at 5^{o}C and 10^{o}C and 30% R.H. Here the seeds should be dried to lower level.

Seed storage sanitation or godowns sanitation

1. Storage environment should be free from insects and rodents
2. Chemicals such as insecticides, fertilizers should not be stored along with seeds.
3. Storage room should be kept cool and dry.
4. Fumigation may be done whenever needed.
5. Use wooden pallets for arranging the bags in cris-cross manner for effective ventilation on all sides of the bags.
6. Seed bags should be stacked up to 6-8 tires depending upon density of seeds.
7. Restacking once in 3 months or less is important for prolonging seed viability.
8. Before storage disinfect the godowns by spraying malathion 50% E.C. @ lit /100 m2 area.
9. If old gunnies, cloth bags and containers are to be used these should be fumigated with aluminum Phosphide.
10. Size of the stack should be 30 x20 feet to facilitate fumigation under gas proof or polythene covers.
11. Periodical inspections should be carried out and control measures to be taken i.e. Malathion 50% E.C. @ lit /100 m2 should be applied in every 3 weeks.

It must be borne in mind that fumigation, particularly repeated fumigation may seriously reduce the vigour and even the germination capacity of seeds. Seeds with m.c. greater than 14% should be dried to below this value before fumigation.

Maintenance of viability in storage

1. Store well mature seeds.
2. Store normal colored seeds.
3. Seeds should be free from mechanical injury.
4. Seeds should be free from storage fungi or microorganisms.
5. Seeds should not have met with adverse conditions during maturation.
6. Storage environment or godowns should be dry and cool.
7. Seeds should be dried to optimum m.c
8. Required R.H. and temperature should be maintained during storage.
9. Seeds should be treated with fungicides before storage.
10. Storage godowns should be fumigated to control storage insects, periodically.

11. Suitable packaging materials should be used for packing.