The Neotia University

A practical manual on Crop Improvement II (*Rabi* crops)

Credits: 2 (1 + 1)

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CONTENT

Practical 1: Emasculation and hybridization techniques in wheat & oat

WHEAT (*Triticum aestivum*) (2n = 42 Hexaploid)

https://www.youtube.com/watch?v=v3JKjRd2acE&t=5s

Family – Poaceae

- The inflorescense of wheat is called Ear and in botanical terms it is called Spike.
- The unit is called Spikelet.
- A floret consists of a lemma and palea, which enclose stamens and a pistil, plus two lodicules that regulate the opening of the flowers and anthers.
- Flowers are incomplete, bisexual and zygomorphic.
- Each floret has three stamens with large anthers & a pistil bearing bifid feathery stigma.
- Wheat stamens are small & produce about 1,000 to 4,000 pollen grains per anther.
- Anthesis start at the middle and these proceeds in both directions. It takes about 2–3 days for a wheat spike to complete.

Much of the pollen grains shed within the floret and the crop is largely self-pollinated. The glumes normally open during the flowering process, the anthers protrude from the glumes and part of the pollen grains is shed outside the flowers. Entry of foreign pollen at flower opening may result in a small extent of cross pollination which is normally less than one per cent.

Selfing

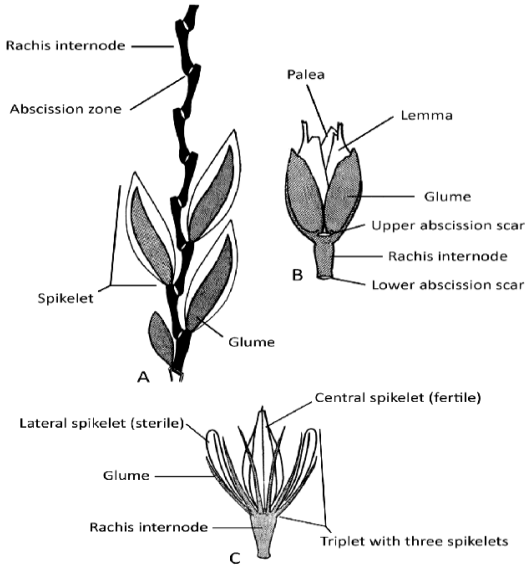
The inflorescence is covered with a butter paper cover prior to anthesis, and kept undisturbed till the flower opening completed.

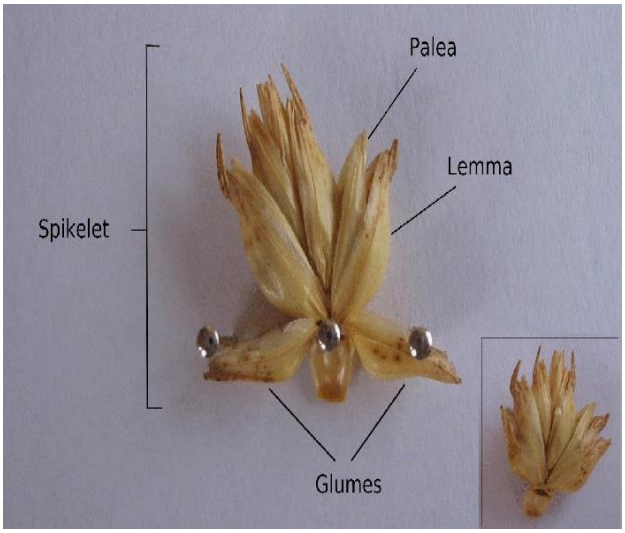
Emasculation: process of removing stamens at bud stage.

- Making a completer flower female; preparation for crossing.
- The Emasculation is done 1–3 days before the anthesis of flower.
- Right Stage-Anthers are of light green but not yellow or cream-colored.
- Unwrapping spike from flag- leaf sheath.
- One to three of the upper and basal spikelets are usually non-functional and are removed with the scissors.: reduces competition for nutrients, eliminates accidental self-pollination of emasculated flowers.
- Clipping lemma and palea before removal of the anthers.
- Removal of three Stamens with the help of scissor.

Pollination

- On the next day ear head selected from the pollen parent are used for crossing. The upper half of the glumes of the few medium spikelets are cut of and the ripened bright yellow anthers are rubbed on the styles of the emasculated florets and then covered.
- Emasculated flowers should be pollinated within 2–4 days, when stigma is receptive with fully developed feathery features.
- Male parent should be in which anthers extrusion has started to get good pollen.
- Forceps are used to remove anthers from the florets.
- Cover bag is removed from the emasculated spike so the pollen can be gently brushed on one or several stigmas OR Top of the bag covering the female spike is cut to allow the male spike to be inverted into the bag, parallel to the female spike. The male spike is then vigorously rotated by twirling the peduncle between the thumb and forefinger.





Precautions

- During emasculation care must be taken not to injure the gynoecium.
- At the time of pollination, one must ensure that the stigma is receptive which is shown by sticky ooze from the stigma.
- Pollen must be collected from freshly dehisced anthers.
- Tagging with proper labels must be attached to every pollinated flower.
- Instruments used for breeding must be clean and sterile to avoid chance pollination.
- Good sanitation should be maintained in and around the area. All the debris and anthers petals etc. should be removed during emasculation and must be disposed of properly.

OAT (*Avena sativa* L.) (2n = 14)

https://www.youtube.com/watch?v=jLHklw8v7qs

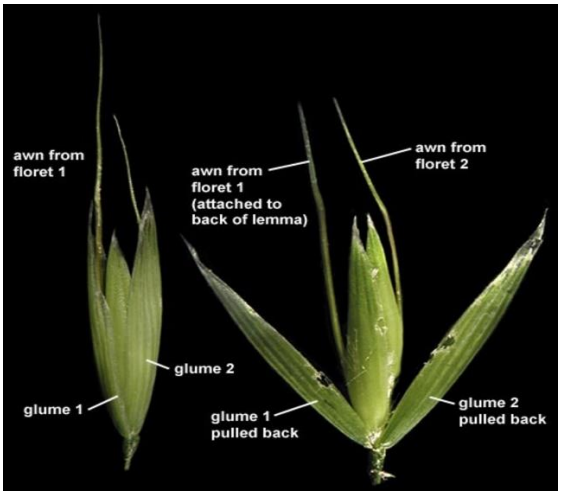
Family: Poaceae or Graminae

- The flower is enclosed in a scales or bracts and grouped in a characteristic structure are called spikelet
- Spikelet of oat are arranged in a panicle
- Each spikelet has a small joints axis or rachilla which bears floret
- Within the spikelet the florets are present.
- Floret may vary from 2 to 3 when it consists of 3 florets, it may consist of
  - opened primary floret
  - unopened secondary floret
  - rudimentary tertiary floret
- Blooming begins in the upper floret.
- Oats has an incomplete flower because it lacks sepals and petals.
- Each floret consists of lemma, palea, stamens and stigma, two large glume.

Oat Crossing Methods

1. Choose male and female parents
2. Emasculate female parent
   - ✓ Choose a female panicle that is just emerging from the flag leaf
   - ✓ Peel back the flag leaf and remove underdeveloped spikelets
   - ✓ Peel back glumes
   - ✓ Remove secondary floret
   - ✓ Fold glumes back over the primary floret
   - ✓ Cut the floret back until you can just see the anthers

- ✓ Remove the three anthers
- ✓ Aim to emasculate 20 florets per panicle
- ✓ Remove any remaining florets that were not emasculated



Pollination

- ✓ Remove anthers from the male parent and dust the pollen on top of the female flower
- ✓ Clip back the male florets to allow pollen to shed
- ✓ Place male and female panicles in dialysis tubing

Exercise:

1. **Prepare the plant material for hybridization in wheat and oat. Make labelled diagrams showing the procedures.**

Practical 2: Emasculation and hybridization techniques in chickpea, cowpea & lentil.

https://www.youtube.com/watch?v=zomjCuYyAJ4

Bengal Gram / Chickpea (*Cicer arietinum* L.) 2n = 16

Family: Leguminosae

Floral Structure

Flowers are solitary, axillary, peduncle is joined, calyx is united with 5 teeth, corolla is 1cm long, greenish white to pink or blue in colour, standard petal is broad and clawed, wings free, keel incurved, stamens 9 + 1, style is filiform and beardless with terminal stigma, pods inflated and 1 or 2 seeded.

Floral Biology

In gram or chickpea, anthesis starts between 9 – 10 am and may continue up to 3 pm. The flowers remain open 2 days. The flowering process will be over early on the second day. The plant is primarily self-pollinated as 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. Cleistogamy has also been recorded in the species.

Selfing and Crossing

The mechanism which promotes self-pollination is cleistogamy and therefore, the self-pollination the sole in this crop, so there is no need to bagging. To have hundred percent selfing, bagging may be done.

a) Buds that will open in one day should be selected for hybridization.
b) The bud is gently held between thumb and fore finger of the left hand, and the standard is just turned above with the help of forceps.

c) Take out all the ten anthers by exposing wings and keels. To ensure that all the 10 are removed, no anthers burst.

d) Observe carefully with magnifying lens for any remaining of anthers and bagging is done.

e) Always dip the forceps in alcohol between the emasculations.

Pollination

- Flowers from the male parents should be picked the morning of anthesis and dust its pollen on the receptive stigma of the emasculated bud.
- If the pollen from these flowers is to be used for late afternoon pollination, they can be refrigerated in separate bags until needed.
- Evening emasculation and morning pollination as well as morning emasculation and immediate pollination have been used. However, morning emasculation and immediate pollination was found to be better.
- The pollen from one bud can pollinate 4 – 6 emasculated buds.
- Pollination should be done in the morning between 8 am and 12 noon. After pollination the date should be recorded on the tag.
- Tags of varying colours may be used to code parents to identify the given crosses such as BC_1, F_1, BC_2.

Cowpea (*Vigna unguiculata*) (2n = 22)

Floral Structure

The inflorescence in an unbranched, axillary raceme bearing several flowers at the terminal end of the peduncle. Peduncle vary from 5 – 60 cm in length and slightly twisted and ribbed. The flowers are in alternate pairs. Flowers are bracteates and caduceus (calyx falls off as soon

as the flower bud open). Calyx is campanulate and 5 teethed, gamosepalous with free upper portion. Corolla contains 5 petals and is papilionaceous. Stamens are in diadelphous (9 + 1) condition. Anthers are uniform and ovary is superior with many ovules.

Floral Biology

There are 2 – 3 flowering periods with interspersed non-flowering periods. The first flowering period is of about 23 days, then non-flowering period of 12 days. It takes 11 – 14 days for the flower to develop and bloom. The flower opening varies within the same varieties *i. e.*, some flowers open at around 6 am others open at 10 am. On cloudy days, the flowers open in the afternoon. Dehiscence of anthers takes place prior to flowering (blooming). It occurs from 10 pm to 1 am. During dark nights, the dehiscence is delayed.

Selfing and Crossing

The cowpea is a self-pollinated crop and the pollination occurs before the flower opens. However, some amount cross-pollination (1 – 10%) occurs depending on the season and activities of pollen vectors but will vary with the cultivar and more particularly with the bumble bees. In order to have, 100% pure seeds, bagging of young buds is essential.

a) Select the inflorescence containing young buds. Flower / buds that will open next day be selected for hybridization
b) Remove all other mature and immature buds.
c) Flowers, showing a coloured corolla 2 to 2.5 times the length of calyx will generally be at the proper stage for crossing.

d) Take the bud between thumb and forefinger with the keeled side uppermost and make incision at the edges of standard. One side of the standard is brought down securing it in the position with the thumb, and same as done with one of the wings which leave the keel exposed.
e) Remove all the stamens with the help of forceps.
f) Bagging is done.

Pollination

a) Pollination is done in the next morning. Flowers from the male parents should be picked in the morning.
b) The pollens are applied on the receptive stigma of the emasculated bud.
c) Regardless of techniques used, a tag should be attached at the of emasculation. After pollination, female parent, male parent and date should be recorded on the tag.

Lentil (*Lens culinaris* Medic.) 2n = 14

Family: Leguminosae

- Lentil is self-pollinating crop due to cleistogamy, with less than 1% cross pollination.
- Pollination normally occurs just before the flower opens.
- The stalked flowers are arranged along an unbranched axis (a raceme).
- The flowers are pale blue, white or pink.
- The flower consists of 10 stamens (9:1 arrangement).
- The pistil consists of the stigma, the style and the ovary, usually with two ovules.

Exercise:

1. **Prepare the plant material for hybridization in chickpea, cow pea and lentil. Make labelled diagrams showing the procedures.**

Practical 3: Emasculation and hybridization techniques in rapeseed & mustard

https://www.youtube.com/watch?v=QvUfEm8RIqw

https://www.youtube.com/watch?v=tYgPLt-6dDc

- Mustard is important oil seed crop, grown in cool season sub tropics, higher elevations as winter crops.
- It is the second most important oilseed in the world as well as in India after groundnut.
- Seeds contain 40 – 45 % oil and 38-41 % protein.
- In Asia, it is mainly grown in China, India, Pakistan and Bangladesh.
- It is grown in as many as twenty-three states in India.
- Indian mustard is a quantitatively photosensitive and basically temperate crop and requiring cool temperature, below 25°C (within agro-climatic conditions) during growth period. Therefore, this crop grown extensively in north eastern and central parts of our country. Mustard oil is considered to be oil that has low saturated fat as compared to other cooking oils.
- It basically consists of fatty acid, oleic acid, erucic acid and linoleic acid.
- It has antioxidant and cholesterol reducing properties. It is also loaded with essential vitamins.
- Though this oil is nutty tasting, it is good for heart and also has many other benefits.

1.	**Name of crop**	**:**	Mustard (Indian Mustard)
2.	**Botanical name**	**:**	*Brassica juncea* L.
3.	**Family**	**:**	Brassicaceae (Cruciferae)
4.	**Chromosome number**	**:**	2n = 36
5.	**Center of Origin**	**:**	Middle East, China
6.	**Mode of pollination**	**:**	Often Cross pollination
7.	**Out crossing percentage**	**:**	4 to 14%
8.	**Related/wild species**	**:**	*B. japonica, B. nigra* (2n=16), *B. oleracea* (2n=18), *B. carinata* (2n=34), *B. carica, B. napus* (2n=38) *B. compestris* (2n=20)

Constraints / causes for low yield potential in mustard:

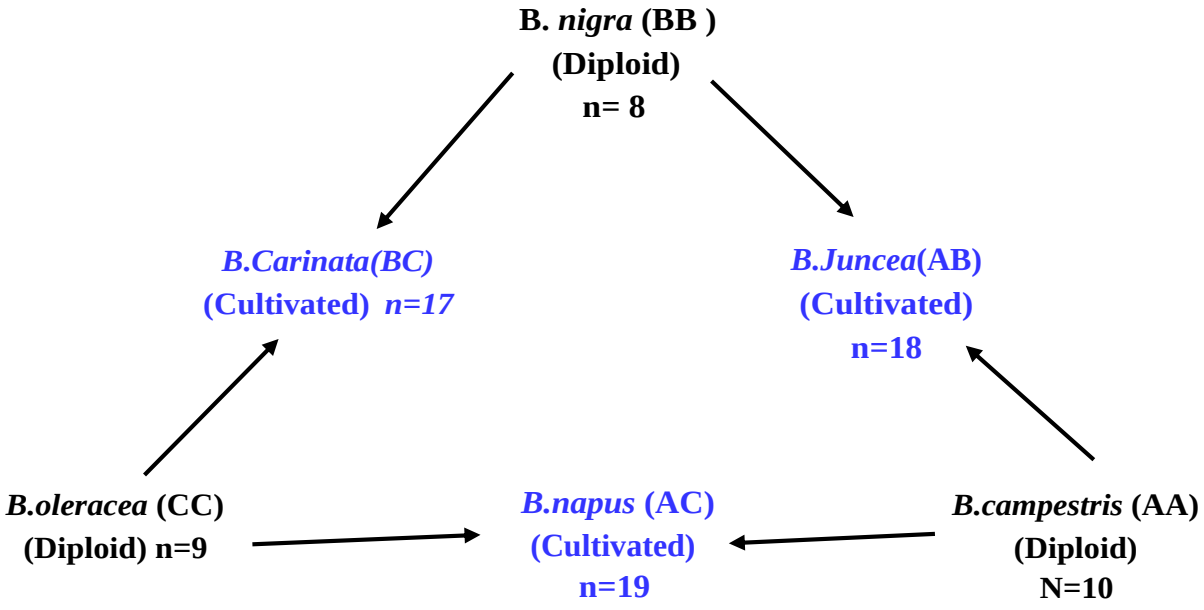
- Mustard is grown in unproductive marginal lands with low levels of inputs and

aberrant weather conditions.

- Continuous adaptation of the traditional package of practices.
- Susceptibility to disease (white rust), pests (aphids) and frost.
- Inadequate production and poor supply of quality seeds of improved varieties.
- The unhealthy fluctuating marketing trends with poor support price.
- Poor water management and inadequate use of fertilizers.
- Genetically, mustard crop has narrow genetic base.

Brassica Triangle:

The genetical relationship between the oilseed brassicas are represented as follows



Genetic origin of Indian mustard (Rai):

Brassica nigra
2n= 16 (BB)

x

Brassica campestris
2n= 20 (AA)

↓

F_1
2n = 2x = 18 (AB)
(Chromosome doubling)

↓

Brassica juncea, 2n= 4x= 36 (AABB)

Allotetraploid or Amphidiploid

Floral Biology:

- The inflorescence is of *raceme* type.
- The flowering is indeterminate commencing from the base towards the tip of the main raceme.
- The buds flower within two hours after sun rise.
- The stigma is generally receptive three days before and after flower opens which mostly lead self-pollination.
- In the bud stage, as flower opens and the time of dehiscing approaches, the inner whorls of the four anthers with longer filaments undergo a twist of 60^{o} to 180^{o} and result in extrorsely dehiscence in the case of self-incompatible types. The outer whorls of the two anthers with shorter filaments do not show this twist and dehisce introrsely.
- The dehiscence of all the anthers in self-compatible types is introrsely.
- Pollination is carried out by wind and mostly by honey bees (*Apis mellifera*).

Androecium:

- The stamens are six, four with long and two with short filaments (Tetradynamous). (Tetra - four, dynamics – strength): Out of six stamens, four inner stamens are long and two outer stamens are short.
- In the bud stage the immature stamens are always below the stigma. Just before the opening of the flower the four stamens, which have long filaments carry the anthers up to the stigma and the anthers are introse. (Anthers which have their face towards the pheriphery of the flower is called extrose anthers. When the face of anther lies towards center of the flower, it is known as introse anther).

Gynoecium:

- The flower bears a hypogynous, syncarpous ovary (Syn – together or united, carpous – fruit or ovary), which is bicapellary with a very large number of ovules and parietal placentation.
- Before the flower opens, the style often increases in length. Generally, the stigma remains in flush with the opening of the tube formed by the corolla.

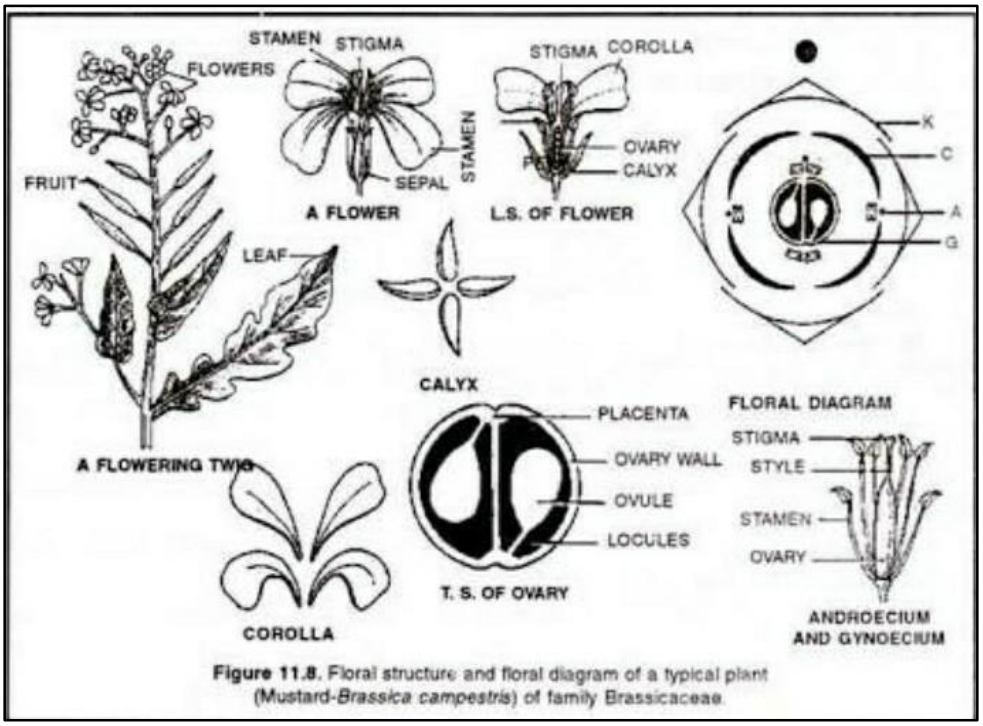


Figure 11.8. Floral structure and floral diagram of a typical plant (Mustard-*Brassica campestris*) of family Brassicaceae.

Anthesis:

- The flowers begin to open from 8.00 a.m. to 12.00 noon.
- The flowers continue to open till 3-4 days and on the 4th to 5th day, the petals and sepals are shed.
- As the flowers open, the anthers begin to dehisce from the apex downwards.
- At the time of dehiscence, the pollen liberating sides of the anthers, remain towards the stigma and slightly shaking of the flowers by wind etc. is sufficient to accomplish the transfer of pollen.
- Large numbers of bees visit the flowers soon after they open and certain amount of cross-pollination takes place.

Selfing technique:

- Selfing is carried out using muslin cloth bags effectively. Either the whole plant or a branch is bagged to ensure self-pollination.

Emasculation:

- When the plant just commences its peak-bloom period, a lateral shoot without any fruit of the first or second order is chosen.
- About six to eight buds, likely to open on the following day or a after that, are left for emasculation and other are removed.
- The stamens are removed with help of fine forceps or with a pair of scissors.
- After removal of stamens, the opened buds are closed gently by rubbing the

forceps along the sepals in an upward direction and cover the emasculated bud by muslin cloth or paper bag.

Crossing technique:

- Ripen anthers from fresh flower are collected in the morning (around 7 am) of the next day and placed under sun rays for dehiscence.
- Pollination is made by dusting pollens to stigma.
- After pollination the flowers are again bagged.

Exercise:

1. **Prepare the plant material for hybridization in rapeseed and mustard. Make labelled diagrams showing the procedures.**

Practical 4: Emasculation and hybridization techniques in sunflower

https://www.youtube.com/watch?v=p5T481baVno

SUNFLOWER (*Helianthus annus* L.) (2n = 34)

Family: Asteraceae

- Sunflower is one of the important oilseed crops grown in India.
- Its genetic name 'Helianthus' is derived from the Greek word 'halio' meaning 'sun' and `anthos' meaning 'flower'.
- It is a crop of the temperate region, it has spread to all the continents of the world like Russia, Bulgeria, Romania, Canada and USA.
- In India, it is extensively grown in Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh.
- Its seed contains 40-42 per cent edible oil with low cholesterol content.
- The oil is also suited for soap making and for preparation of a number of other allied products.
- The oil cake contains 40-44 per cent protein.
- Sunflower meal is used as a bird and animal feed.
- Sunflower oil is considered relatively good quality oil in comparision to most other vegetables oil because of its light colour, bland flavour, high smoke point, high level of oleic acid and absence of linolenic acid.
- The unsaturated fatty acids, oleic and lenoleic comprises about 90% of total.

Floral biology:

- The disc-shaped head called capitulum (diameter varies from 6 to 75 cm) is borne terminally on the main stem and branches.
- The capitulum consist of an outer whorl of showy and generally yellow ray florets which are normally sterile, having rudimentary pistil and vestigial style and stigma, but no anther.
- The main function of ray florets is to attract honeybees and pollination.
- Flowers over the reminder of the discoidal head are called disk florets (number varies from 700 t0 3000) which are perfect flowers and epigynous in nature.
- The corolla of each floret has 5 fused petals except at the tip. Inside the corolla tube, 5 fused anthers form as second tube which encloses style which termites distally in a bilobed stigma curls outward above the anther tube.
- Flowering starts from the periphery to the centre of the capitulum.

- Suflower is protoandrous in nature and there is 10 to 12 hrs difference in maturation of male and female elements.
- Honeybees play an important role in cross-pollination.

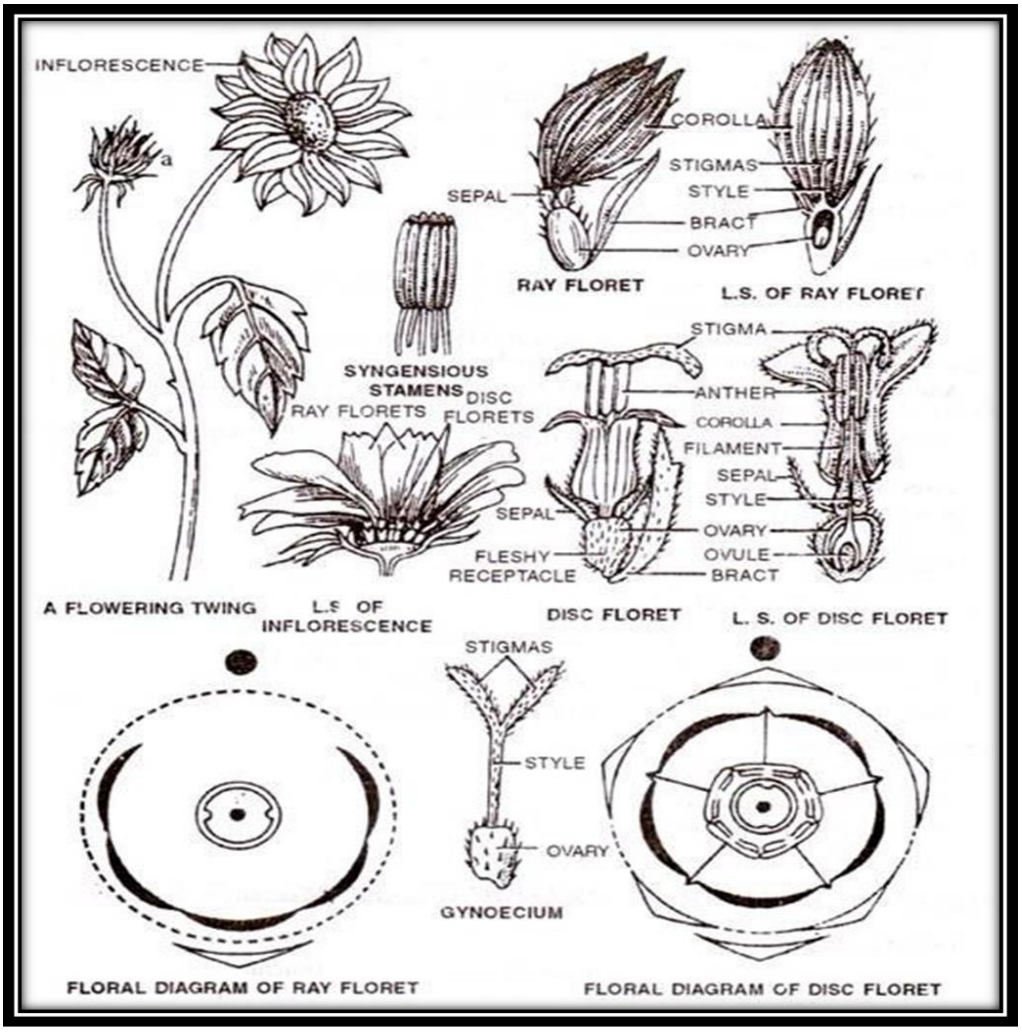
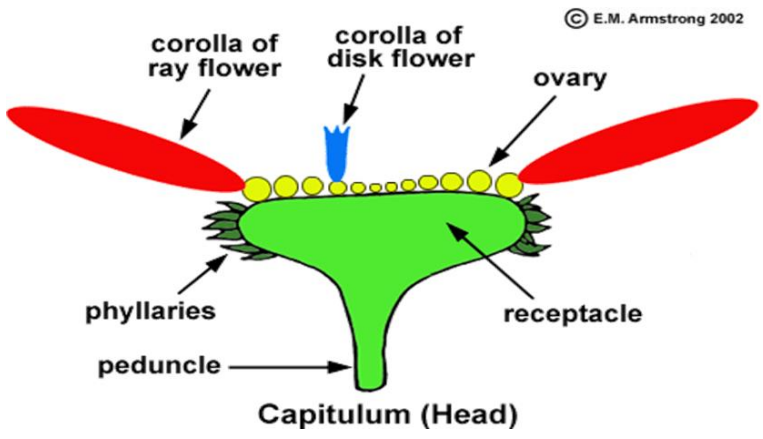


Figure 4.1: Floral structure of sunflower



Selfing techniques

Selfing is done by bagging of the entire head. The bagging material could be cotton cloth or butter paper bag.

Crossing techniques.

1. **hand emasculation**:

- Emasculation is done by removing the anther tubes with forceps on the morning that the flowers open. Un emasculated flowers are removed.

2. **Chemical induction of male sterility:**

- This is achieved by spraying of 0.05% solution of gibberellic acid per plant during bud initiation.
- Application of gibberellic acid after bud initiation consecutively for 3 days in the morning gives better results.

3. **pollination:**

- Pollination is carried out by collecting the pollen from the male parent which are already bagged prior to flowering. Pollen can be collected from the flowering head. Pollination is usually done in the same morning after emasculation

Exercise:

1. Prepare the plant material for hybridization in sunflower Make labelled diagrams showing the procedures.

Practical 5: Emasculation and hybridization techniques in potato

https://www.youtube.com/watch?v=bcEkXhC5Aqs

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

Family: Solanaceae

- Potato (*Solanum tuberosum* L.) is the most important non-grain food crop in the world, ranking 4th in terms of total food production rice wheat and maize.
- It is grown in around 150 countries spread across both temperate and tropical regions and at elevations from sea level to 4,000 m.
- More than half of the potato production takes place in developing countries including India, and over one billion people have potato as their staple diet.
- Nutritionally, potatoes are second only to soybean for amount of protein/ha, with the major storage protein being patatin, one of the most nutritionally balanced plant proteins known.
- The lysine content of potato complements cereal based diets that are deficient in this amino acid.
- A single 150g tuber provides up to 45% of recommended daily allowance (RDA) for vitamin C, 10% vitamin B6, 8% niacin, 6% folate as well as significant amounts of other essential mineral nutrients required.
- Diversified uses of potato covers fresh food, processed products, animal food, seed and raw material for industries (mainly starch as raw material for alcohol, dextrin and glucose).

Floral Biology:

- The inflorescence of the potato is cymose and may be simple or compound.
- The potato flowers are bisexual and possess all the four essential whorls.
- Calyx is composed of five sepals united at the base to form bell shaped structure below the carolla.
- The corolla consists of petals joined at the base and forms a short tube with a flat five- lobed surface. The colour of corolla may be white, light blue, blue, red, or purple with different combinations and intensities. The colour of the corolla is valuable trait in distinguishing different varieties.
- Androecium consists of five stamens. The stamens are composed of anther and filament joined to the corolla tube. Anthers are fused in a conical structure or spread loosely enclosing the pistil. The colour of anther varies

from light yellow to deep orange. Pollens dehiscence is through the pores.

- Two carpels fuse to form as syncarpous, bilocular, superior ovary with long style and stigma with 2 lobes.
- Flowers in the cultivated potato open mostly in early morning.
- Germination of the pollen is completed after 30 minutes and the ovary is fertilized within 12 hours. Cross pollination is most often accomplished by bumblebees.
- The mature fruit is a green berry with axial plancentation.The cultivated tetraploid potato species (*S. tuberosum sub* spp. *tuberosum* and *S. tuberosum sub* spp. *andigena*) are photoperiodically long day plants and require long days and short nights for their flowering, but more than 90 per cent of the potato growing area in India is in plains and in *rabi* season (October planting) when the day lengths are shorter. Therefore, the flowering does not take place in the plains.

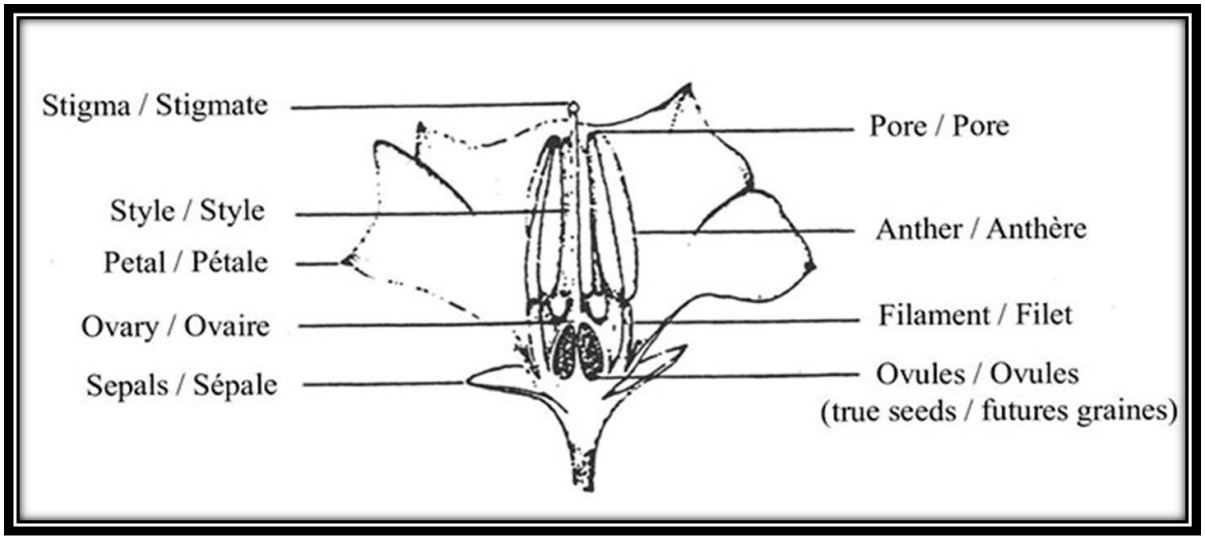


Figure 5.1: Floral structure of potato flower

Emasculation:

- In female parents about 4-5 flower buds per branch are retained by removing very small and old flowers as well as formed berries.
- In the process of emasculation, anthers, petals and half portion of sepals is removed from the unopened fresh flower buds. Care is taken to injure stigma during the course of emasculation.

Crossing technique:

Pollen collection:

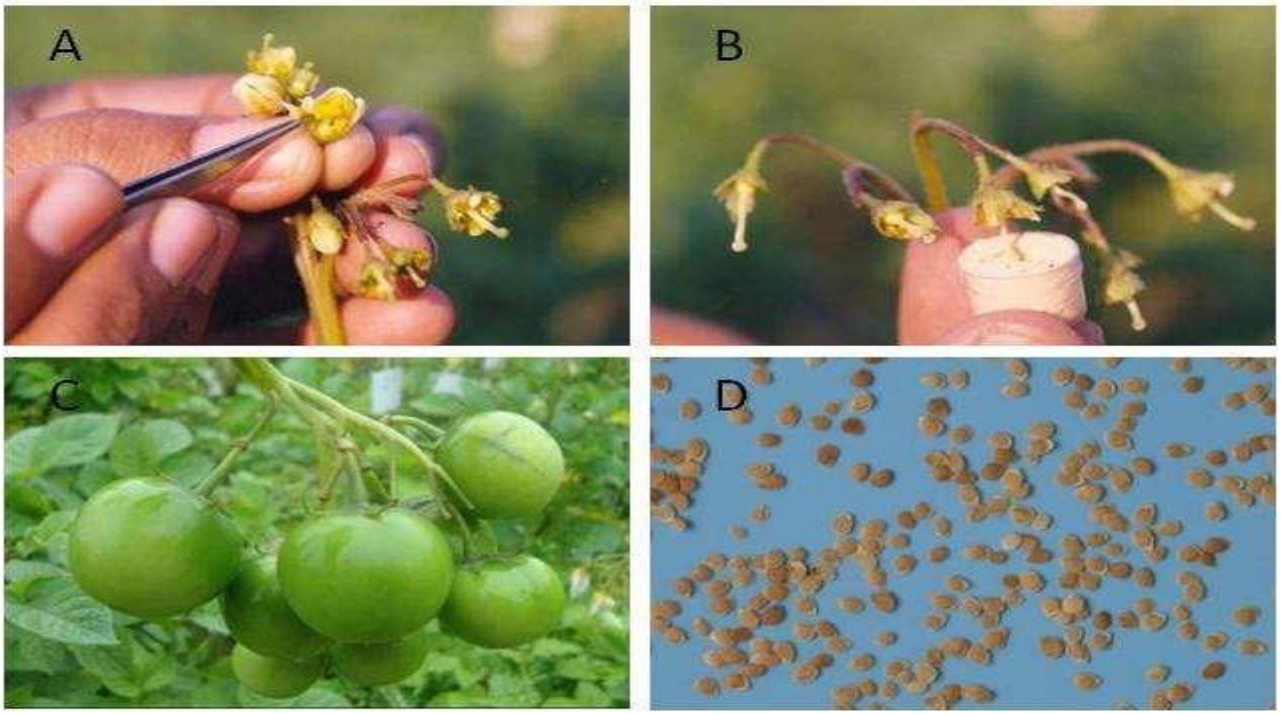
- Flowers from male parents are collected in the evening preceding the day of

pollination.

- Freshly opened flowers with anthers that are about to shed pollens or large sized bud, which would open next day should be collected and spread on a sheet of paper placed on the table at room temperature.
- Stigma and petals are removed from the male flowers, so that pollen does not stick to these while pollen extraction.
- Next day morning, anthers are separated and pollens are extracted shacking anthers in nylon tea sieve.
- The can be stored in a refrigerator at 6-8^{o} C for future.

Pollination:

- Each receptive stigma is pollinated by dipping the tip of stigma in pollen.
- Repeated pollination of receptive stigma twice or thrice at interval of 8 hrs is known to produce higher percentage of berries per flower bunch and more seed per berry.
- The pollinated bud is tagged with label with information like cross details, number of buds pollinated and date of pollination is noted in the hybridization register.



Exercise:

1. Prepare the plant material for hybridization in potato. Make labelled diagrams showing the procedures

Practical 6. Emasculation and hybridization techniques in sugarcane.

https://www.youtube.com/watch?v=iqkOxvsZqEs&t=25s

The word sugarcane is derived from Sanskrit word 'sharkara' meaning sugar. It includes 3 cultivated species like *S. officinarum*, *S. barberi* and *S. sineese*. Sugarcane is an important sugar crop in all the countries of tropical Asia. Sugarcane is grown in more than 110 countries. The world's largest producer of sugarcane is Brazil, followed by India, China, Thailand, Pakistan, Mexico, Columbia, Australia, America and Philippines. The sugar industry is the second largest agro-based industry in India in terms of economic gains and employment potential. The states having substantial sugarcane area in India are, Utter Pradesh, Maharashtra, Haryana, Andhra Pradesh, Tamil Nadu, Karnataka, Bihar and Punjab.

Sugarcane products include table sugar, molasses and ethanol. The bagasse that remains after sugarcane crushing may be may be burned to provide heat and electricity. Because of its high cellulose content, serve as raw material for making paper and cardboard. In Brazil, ethanol extracted from sugarcane is blend with petrol for running vehicle.

1.	**Name of crop**	:	Sugarcane
2.	**Botanical Name**	:	*Sachharum officinarum* L.
3.	**Family**	:	Poaceae
4.	**Chromosome Number**	:	2n = 80
5.	**Centre of Origin**	:	New Guinea and North India
6.	**Mode of pollination**	:	Cross pollination (protogyny)
7.	**Out crossing percentage**	:	42-50 %

Floral biology: -

Photoperiod is an important factor for flowering in sugarcane. It flowers all the year round at equator, where day length is constant for 12 hours. Warm nights, humid conditions and high rainfall favours flowering, white cool weather and high altitude where the day length varies affecting the flowering.

- The inflorescence / panicle is just like arrow shape hence it is called arrow, contains flowers about 10,000 to 50, 000 individual spikelets.
- The flowers are in pair, one sessile (without petiole) and one pedicellate (with petiole). The sessile spikelet flowers before the pedicellate spikelet.
- The stamens are three and stigma is feathery and bifurcated.

- The flowers open in the morning between 5 and 6 a.m. About 7 to 14 days are required for an arrow to complete the flowering.
- The flowers show a wide range of fertility, ranging from male sterility to high pollen fertility. Generally, the flowers are protogyny in nature.
- A mature sugarcane seeds consists of the mature dry fruit (caryopsis), glumes, callus hairs, anthers and stigma which are extremely small in size, often poorly developed and invisible with sticky hairs called fluff. Fluff is the entire flower panicle without the main flower axis and larger lateral axes used for breeding programme.

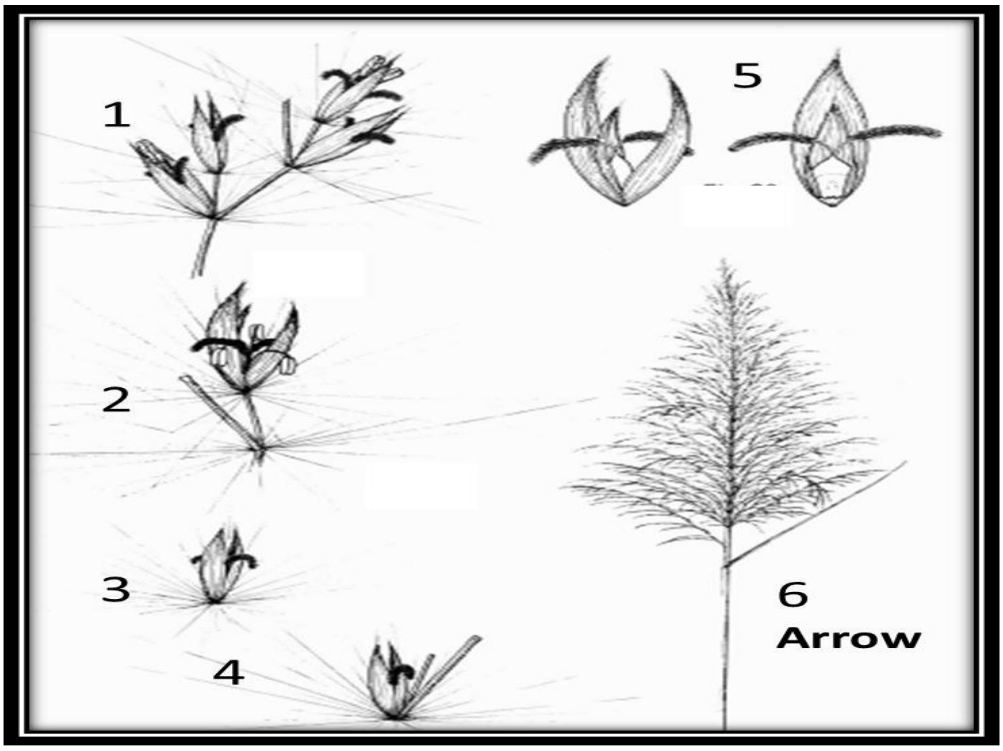


Figure 7.1: Diagram of matured inflorescence of sugarcane showing developmental stages of maturing spikelet

Selfing technique:

- The arrows of sugarcane are covered in a lantern (A bamboo frame work or cage like structure which covered a muslin cloth bag or a polyethylene) before anthesis to ensure selfing.

Crossing technique:

- Due to small and large number of flowers on arrows and tall plant height emasculation is not practicable.
- The female parent is selected on the basis of protogyny, male sterility or late pollen

shading.

The crossing is attempted by any of the following three methods:

- The male and female parent is planted closely. The male and female arrows are enclosed in a single lantern.
- The arrows from male parent are cut-off and cut end of arrow is kept in weak sulphur dioxide solution (arrow can be kept alive) and then these arrows are dusted on female arrows.

Marocotting procedure (technique):

a. This procedure was first used India and now it is adopted by several countries. The procedure in which a plastic sleeve containing a growth medium is secured about 2 and 3 nodes above the base of the stalk to induce rooting is called marcotting. In this technique, healthy female clones are selected.
b. Just prior to flowering, a polyethylene strip containing a mixture of moist potting soil is wrapped around a bud of the sugarcane stalk about two nodes above the ground level.
c. Roots will develop on the stalk within 10 days where the bud has been marcotted.
d. The cane is cut below the rooted portion and taken to the crossing area, where they are transplanted in a big plots containing soil.
e. Clusters of such 3-4 female arrows are usually kept in a lantern and pollination is carried out either by enclosing male and female arrows under same lantern or by dusting the pollen.

Coimbatore method

During flowering period, the sugarcane stem will be cut leaving one or two bud. The cut stem can be transferred to a mud pot having moist mud. Within 10 days the buds will develop into roots and there will be good root system. This can be transferred to the breeding block. In the crossing block, the male and female plants are covered with common lantern. Free shedding pollen over female plant will occur. We can harvest both selfed and crossed seeds from the female parent. The selfed seeds can be identified by chromosome number by raising it in the nursery. Selfed seeds thus removed retaining crossed seeds.

Lantern method

Providing Lantern for a female plant before anthesis starts. From the desired male parent cut the arrow. That arrow can be introduced into the Lantern and shaken up and thereby crossing can be effected. This will be repeated for 2-3 days in order to have more seed set.

Hawaii method (Sulfurous acid Technique)

A sulfurous acid solution keeps the inflorescence alive for several weeks. Here, we cut both male and female flowering arrows along with small portion of stem. These cut end will be immersed in a vessel containing 0.01% sulphuric acid and 0.01% phosphoric acid. The cut end at the lab is brought nearer and effect cross pollination.

Flowering is undesirable in commercial canes: -

- Plant stops growing and matures rapidly after the appearance of the flowering stalk.
- Reduction in cane and sugar yield as the food is diverted towards growing inflorescence.
- Sugarcane stalk becomes hollow.

Exercise

1. Prepare the plant material for hybridization in sugarcane. Make labelled diagrams showing the procedures.

Practical 7: Emasculation and hybridization techniques in onion.

https://www.youtube.com/watch?v=kAu_49kWOBE

ONION (*Allium cepa*) 2n =2x=16

Family: Alliaceae

- Onion is one of the oldest vegetables grown throughout the world.
- A global review of major vegetables show that onion ranks second after tomato in area.
- The leading onion growing countries are China, India, USA, USSR, Japan, Spain, Brazil, Italy, Egypt and Pakistan.
- In India, it is grown in Maharashtra, Gujarat, Andhra Pradesh, Bihar, Karnataka, Madhya Pradesh, Orissa, Tamil Nadu, Uttar Pradesh and West Bengal.
- Onions are used in a large number of recipes and preparations spanning almost the totality of the world's cultures.
- They are now available in fresh, frozen, canned, pickled, powdered, chopped and dehydrated forms.
- Onions can also be used, usually chopped or sliced, in almost every type of food, including cooked foods and fresh salads and as a spicy garnish.

Two types of onions commercially grown in India:

1. **Common onion** (*Allium cepa* var. *cepa*)**:** This is most important in commercial trade. Bulbs are large, normally single and plants reproduce through seeds.
2. **Multiplier onion** (*Allium cepa* var. *aggregatum*)**:** This produces bulbs of smaller size and they are several in numbers to form an aggregated cluster. Propagation is usually vegetative via daughter bulbs.

Floral Biology:

- The flowering structure is called an umbel forms aggregate of small inflorescences (cymes) of 5 to 10 flowers, which open in a definite order and to last for 2 or more weeks.
- The individuals flower contains 6 stamens, 3 carpels, united into one pistil and 6 perianth segments. The pistil contains 3 locules each of which has 2 ovules.
- The flower is protoandrus. Anthers shed pollen over a period of 3 -4 days prior to stigma becomes receptive leading to cross-pollination.

Bolting: When an onion plant produces a flower stalk is called as bolting.

Selfing technique:

In onion, selfing beyond S2-S3 generation is very difficult due to high inbreeding depression. Mostly selfing is done by sib-mating (rubbing umbels against each other) in cages or three ring muslin cloth bags are used for selfing.

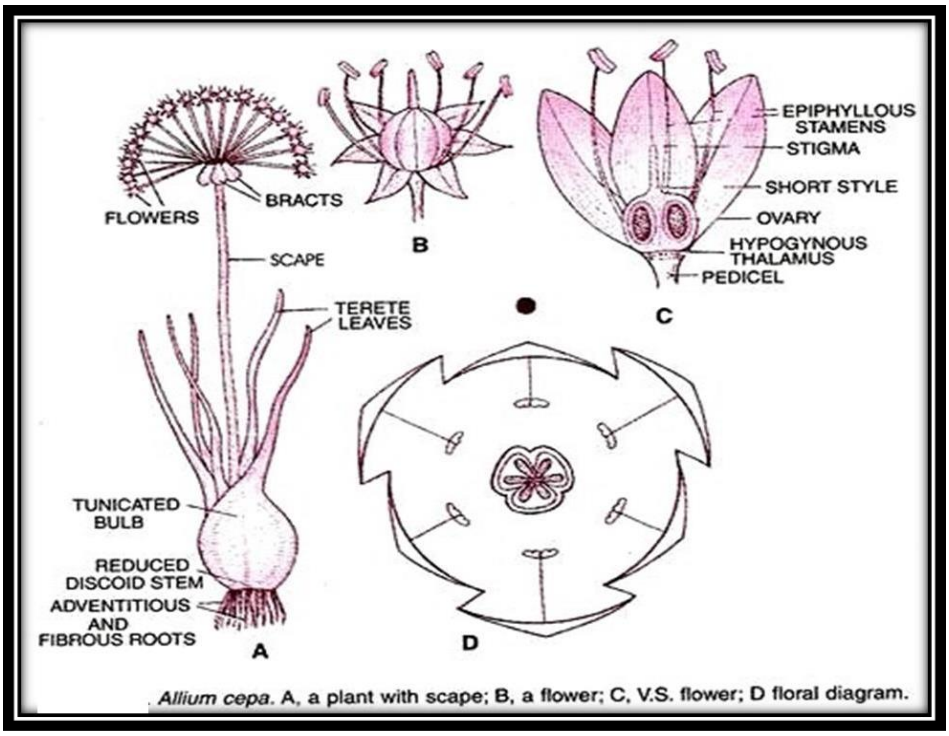


Figure 11.1: Plant and floral structure of onion

Emasculation:

- As soon as few buds in an umbel open, the whole umbel of the female parent is bagged in a muslin cloth bag. The flowers are removed daily for a few days until the peak flowering has reached after which buds are emasculated as they open.
- When sufficient buds have been emasculated, the remaining young flower buds are removed.

Crossing Technique:

- The umbel of pollen parent covered by a muslin cloth bag is cut off and its stalk placed in a glass bottle filled with water.
- This bottle is fastened to a bamboo/wooden stake and fixed in soil close to the female parent. Female parent umbel (emasculated one) and the pollen parent umbels are now enclosed in the same common bag.
- For few days in the morning, the male umbel is gently rubbed over the emasculated umbel to ensure pollen shedding and cross-pollination.
- A few common houseflies can also be introduced into the bag for pollen transfer.

Exercise:

1. Prepare the plant material for hybridization in onion. Make labelled diagrams showing the procedures.

Practical 8: Emasculation and hybridization techniques in brinjal and tomato

https://www.youtube.com/watch?v=cvDcijYJ7BQ

BRINJAL (*Solanum melongena* L.) (2n =2x= 24)

Family: Solanaceae

- The brinjal also knows as "Egg plant".
- Brinjal, tomato and pepper are the member of the nightshade family.
- It is a mainly grown in plains and is available more or less throughout the year.
- In India brinjal is cultivated throughout the country and liked by both poor and rich.
- It is primarily used as cooked vegetable and popular for the preparation of various dishes in different regions of the country.
- It is rich source of vitamin A and B. It has also medicinal value in *Ayurveda*.

Floral biology:

- The flower is bisexual and it contains stamens that dehisce at the same time when the stigma is receptive, thus, self-pollination is the rule. However, upto 30-40 % cross- pollination is also reported.
- Inflorescence is often solitary but sometimes it constitutes a cluster of 2-5 flowers. Flower is complete, actinomorphic and hermaphrodite.
- Calyx is five lobbed, gamosepalous and persistence, it forms a cup like structure at the base.
- Corolla is five lobbed gamopetalous (characterizing a corolla with partially or wholly fused petals) with margin of lobes incurved.
- Anthers are cone shaped, free and with apical dehiscence
- Anthers start dehiscence from 7.30 a.m. to 11.00 a.m.
- Ovary is hypogenous (having a superior ovary and other floral organs attached bwelow the gynoecium on the receptacle), bicarpellary, syncarpous (gynoecium has multiple carpels "fused" into a single structure) and with basal placentation.
- Stigma receptivity is highest during anthesis i.e. flower opening. The receptivity of stigma can be observed from its plump and shiny appearance which gradually becomes brown with the loss of receptivity.
- Stigma becomes receptive until about four days from flower opening.

Types of flowers depending upon the length of the style.

a. Long style with big sized ovary.

b. Medium styled with medium rudimentary ovary which do not produce fruits.
c. Pseudo- short styled with rudimentary ovary which do not produce fruits.
d. True short styled with very rudimentary ovary which do not Produce fruits.

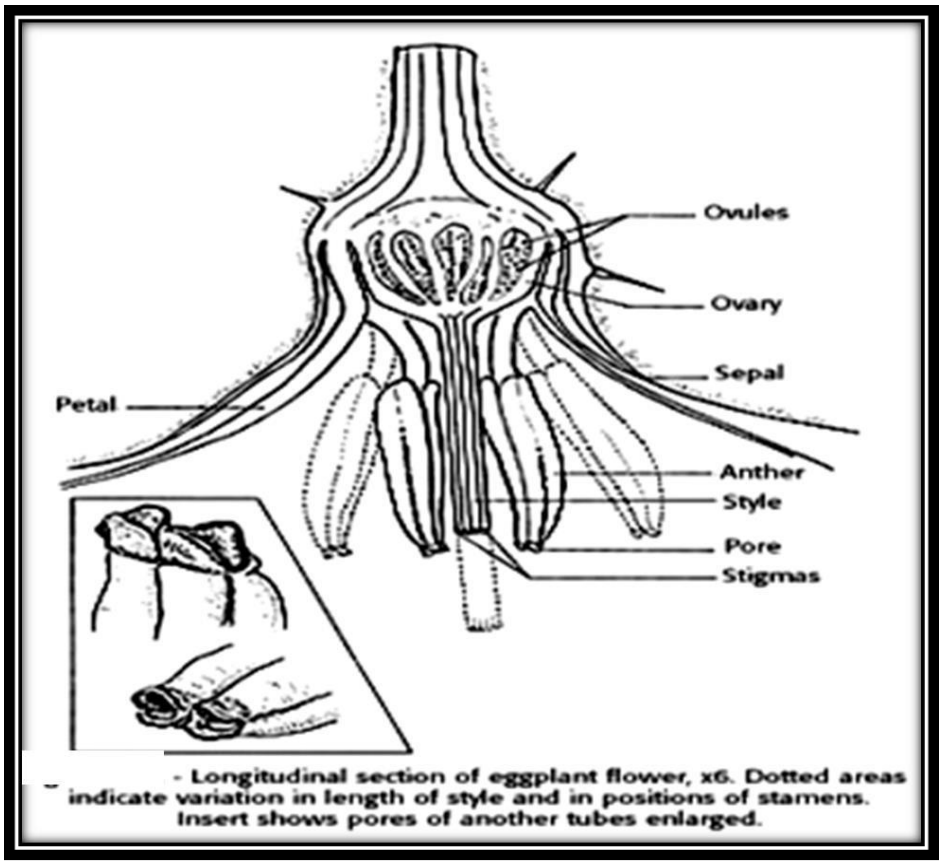


Figure 9.1: Flower structure of brinjal crop

Selfing technique:

- For effective and cent per cent selfing, entire plant or branches of plants are covered with muslin cloth bags.

Emasculation:

- A healthy long styled, well developed bud from the central portion of the plant is selected.
- The bud is opened gently with the help of fine pointed forceps one or two days before the opening of the bud and all the five anthers are carefully removed.

Crossing techniques:

- Freshly dehiscing anthers are picked up and are slit vertically with fine needle to get sufficient pollen at the tip of the needle.
- Collected pollens are applied on stigma of emasculated flower.
- Pollinated flowers are labelled and covered with small pollination bag.


TOMATO *Solanum lycopersicon*, Formerly *Lycopersicon esculentum* 2n =2x=24

https://www.youtube.com/watch?v=9kdqDc3fHEI

Family: Solanaceae

- Tomato is one of the most important vegetable crops grown throughout the world. The leading tomato growing countries in the world are the USA, Several European countries, Japan and China.
- China is the major producer of tomato followed by US, Turkey, India and Italy.
- World acreage of tomato is more than 3 million hectares. In India tomato is grown in about 6.0 lakh hectares.
- It is a rich source of vitamins and minerals. Tomatoes are consumed as fresh and in processed form.

Flower Biology:

- Tomato flowers are bisexual, consist of united sepals at base.
- Corolla is bright yellow in colour and gamopetalous,
- Androecium consist of 5 stamens with small filaments and large anthers and form a solid cone enclosing the pistil. Anther dehiscence is introrsely longitudinal.
- Gynoecium consist of superior, bicarpellary and syncarpous ovary, with single style and bilobed stigma.
- Anthisis starts in morning from 6.00 hrs and continuous till late morning (maximum between 7.00 to 9.00 hrs.
- Stigma receptivity is from 16-18 hours before anthesis and 5 to 6 days after the anthesis
- Pollen remains viable from 2 to 5 days (18-25 ^{o}C).

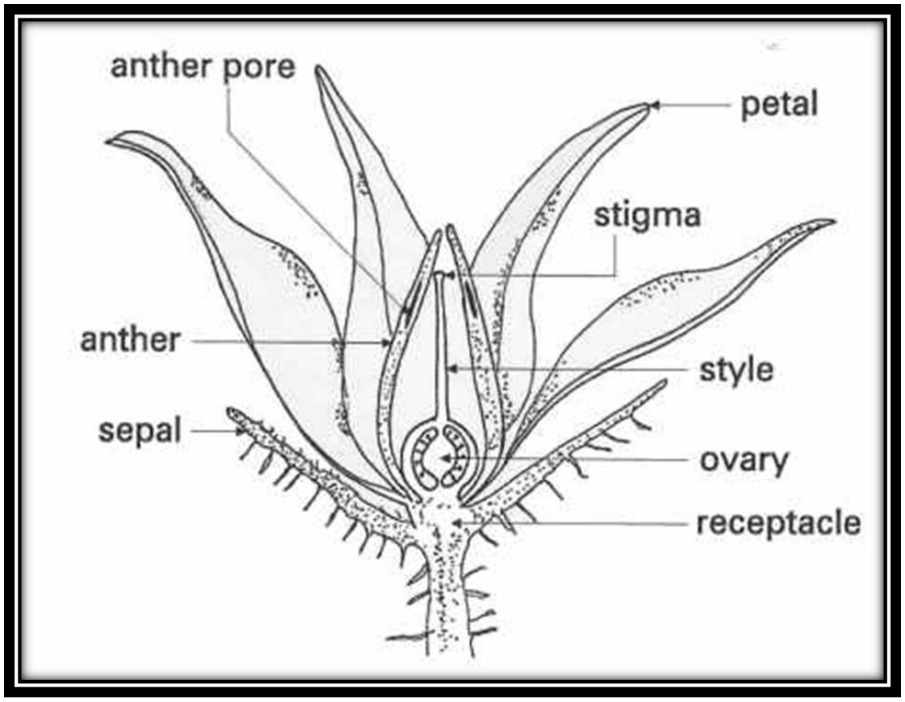


Figure 8.1: Flower structure of tomato crop

Selfing technique: For selfing covers the flower with butter paper bag.

Emasculation:

- Emasculation is usually done in afternoon one day prior to anthesis/flower opening, when anthers and corolla are beginning to change from light to dark yellow. The stigma is fully receptive at this stage allowing for pollination even immediately after emasculation.
- Anthers are removed as a group with or without the surrounding corolla, by inserting forcep between the sepals to grip the base of the anthers and/or petals which are then removed by a firm but steady pull.

Crossing technique:

- Next day forenoon, pollens are collected on the tip of forcep by slitting the forcep inside the anthers of mature flowers and then be lightly applied to the stigmatic surface and should be visible as a white covering.
- Forceps should be sterilized by dipping in alcohol or methylated spirit after each pollination. Protection of pollinated flowers by wrapping with cotton or small pollination bags is essential.

Exercise:

1. Prepare the plant material for hybridization in brinjal and tomato. Make labelled diagrams showing the procedures.

Practical 9: Handling of germplasm and segregating populations by different methods like pedigree, bulk and single seed decent methods.

https://www.youtube.com/watch?v=x7XAoj8eyYg

Mass selection and pure line selection cannot be applied to segregating population. E. g. F_2, F_3 etc. The method is generally used for handling segregation generation may be grouped into three categories.

i) Pedigree Method

ii) Bulk Method

iii) Back Cross Method

The objectives of all these methods are to develop pure line varieties.

In pedigree method, individual plants are selected from F2 and the subsequent generation and their progenies are tested. During the entire operation, a record of the entire parent's offspring relationship is kept, is known as pedigree record. The selection of individual plant is continued till the progenies show no segregation. At this stage, selection is done among the progenies, because there would be no genetic variation within progenies.

Pedigree method of plant breeding includes following steps.

HYBRIDIZATION:

Crossing between selected parent plants is the first step in pedigree method.

F1 generation:

Seeds obtained by hybridization (F1 seeds) are planted with proper sowing distance. Seeds of about 20-30 plants are harvested in bulk and forwarded to grow F2 generation.

F2 generation:

Selection is the main process carried in this step. About 10,000 plants are grown from F1 generation seeds (F2 seeds). With application of selection process about 500 plants are selected and harvested separately.

F3 generation:

About 30 or more progenies are raised from each of the selected plant of F2 generation. About 100-400 superior plants (the number could be anything, preferably less than those selected in F2 generation) are selected.

F4 generation:

Seeds from F3 generation are space planted. Plants with desirable characters are selected in number much less than those selected in F3 generation.

F5 generation:

Individual plant progenies planted in multi row (3 or more) plots so that superior plants (about 50 – 100) can be selected by comparison.

F6 generation:

Individual plant progenies planted in multi row (3 or more) plots. Plants are selected based on visual evaluation, progenies showing segregation can be eliminated.

F7 generation:

Preliminary yield trials with minimum 3 replications and a check. Quality tests are conducted.

F8 to F12 generation:

Multi-location yield trials with replications are conducted. Tests for quality and disease resistance are conducted.

F10 or F13 generation:

Seed multiplication for distribution.

Merits of pedigree method are listed below

Excellent method for improvement of easily observable, high heritability characters. As pedigree record is maintained, information regarding inheritance pattern of characters can be obtained as and when required. Each plant can be traced back to its parent plant. Only those progeny lines which contain plants with desired characters are selected for next generation. So there is scope for plant breeder's skills. Progeny tests are done; thus it is based on genotypic value rather than phenotypic value. Increased breeding efficiency by early identification of superior heterogeneous populations Scope for transgressive segregation to occur for the characters like yield. New variety development takes short period as compared to bulk method.

Demerits of pedigree method are as follows

Costly, labour intensive, requires skilled person as selection is practiced Pedigree record maintenance is time consuming. Selection for yield or other characters in F2 and F3 is ineffective. Selection for yield or other characters in F2 and F3 is ineffective. One important to note is genetic variation available for selection gets decreased in later generations due to the individual plant selection carried out earlier.

Application of Pedigree Method:

1) Selection of desirable plants from the segregating population in self- pollinated crops.
2) This method is commonly used to correct some specific weaknesses of an established variety (Combination breeding).
3) It is also used in the selection of new superior recombinant type's *i.e* Transgressive breeding.
4) This method is suitable for improving specific characteristics such as disease resistant,

plant height, maturity etc.

Handling of segregating generation - Bulk Population Method

Bulk population method of breeding in self –pollinated crop is also known as mass method or population method of breeding. It was first used by Nilsson Ehle in 1908. It refers to a species is grown in bulk plot (from F1 to F5) with or without selection, a part of the bulk seed is used to grow the next generation and individual plant selection is practiced in F6 or later generation. In this method duration of bulking may vary from 6-7 to 30 generation.

Application of Bulk Population Method:

This method is suitable and most convenient for handling the segregating generation of cereals, smaller millet, grain legume and oilseeds. This may be used for three different purposes.

i) Isolation of homozygous lines.

ii) Waiting for the opportunity of selection.

iii) Opportunity for natural selection to change the composition of the population.

Procedure of Bulk Population Method:

1) Hybridization:

Parents are selected according to the objective of the breeding programme and crossed.

2) F1 Generation:

The F1 generation (10 to 25 F1) is space planted and harvested in bulk.

3) F2 - F6 – Generation:

F2 to F6 generations are planted at commercial seed rate and spacing. These generations are harvested in bulk. During these generations the population size should be as possible, preferably 30 to 50 thousand plants should be grown in each generation.

4) F7 Generation:

About 30 – 50 thousand plants are space planted and out of this only 1000 to 5000 plants with superior phenotypes are selected and their seeds harvested separately. Selection is made on the basis of phenotypes of plants, grain characteristics etc.

5) F8 Generation:

Individual plant progenies are grown in single or multi row plots. Most of the progenies would be homozygous and are harvested in bulk. Weak and inferior progenies are rejected and only 100- 300 individual plant progenies with desirable characters are selected.

6) F9 Generation:

Preliminary yield trial is conducted along with standard variety as check. The evaluation of progeny is done for important desirable characteristics. Quality test may be conducted to reject the

undesirable progenies.

6) F10- F12 Generation:

Replicated yield trails are conducted at several locations using standard commercial varieties as check. The lines are evaluated for important agronomic characteristics. If lines are superior to the standard check, released as new varieties.

7) F13 Generation:

Seed multiplication of the newly released variety for distribution to the farmers.

Merits of Bulk Population Method:

1) This method simple, convenient and inexpensive.

2) Little work and attention is required in F2 and subsequent generation.

3) No pedigree record is to be kept.

4) It eliminates undesirable types and increases the frequency of desirable types by artificial selection.

5) It is suitable for studies on the survival of genes and genotypes in populations.

6) There are greater chances of isolation of Transgressive segregates than pedigree method.

Demerits Bulk Population Method:

1) It takes much longer to develop a new variety.

2) It provides little opportunity for the breeder to exercise his skill in selection.

3) A large number of progenies have to be selected at the end bulking period.

4) Information of inheritance of characters cannot be obtained like that of pedigree method.

Achievement:

This method has between used in Barley crop for developing some varieties from the crosses (Allas X Vaughn), like Arival, Beecher, Glacier, etc. In India only one variety "Narendra Rai" has been developed in Brown Mustard. This method has a limited application in practical plant breeding.

Handling of segregating generation – Single seed descent Method

Another modification of the bulk method is the single-seed-descent method, which is becoming increasingly popular. In this method, a single seed from each of the one to two thousand F2 plants is bulked to raise the F3 generation. Similarly, in F3 and the subsequent generations one random seed is selected from every plant present in the population and planted in bulk to raise the next generation. This procedure is followed till F5 or F6 when the plants would have become nearly

homozygous. In F5 or F6, a large number (1 to 5 hundred) of individual plants are selected and individual plant progenies are grown in the next generation. Selection is done mainly among the progenies, and the number of progenies is sufficiently reduced to permit replicated trial in the next generation. Individual plants may be selected only from outstanding families not showing segregation. Thus preliminary yield trials and quality tests begin in F7 or F8 and coordinated yield trials in F8 or F9.

The objective of single-seed-descent method is to rapidly advance the generations of crosses; at the end of the scheme, a random sample of homozygous or near homozygous genotypes/lines is obtained. F2 and the subsequent generations are grown at very high plant densities as vigour of individual plants is not important.

In each year, 2-3 generations may be raised using off-season nurseries and greenhouse facilities. The important features of this scheme are: (1) lack of selection, natural or artificial, till F5 or F6 till the population is reasonably homozygous, and (2) raising of F3 and later generations from a bulk of one seed from each F2 and the subsequent generation plant in order to ensure that each F2 plant is represented in the population.

As a result of the speed and economy, the single-seed -descent scheme is becoming increasingly popular with the breeders. The single-seed-descent scheme

1. advances the generation with the maximum possible speed in a conventional breeding method
2. requires very little space, effort and labour
3. Makes the best use of greenhouse and off-season nursery facilities
4. ensures that the plants retained in the end population are random sample from the F2 population.

However, (1) it does not permit any form of selection (which is implied in the scheme) during the segregating generations; and (2) in each successive generation, the population size becomes progressively smaller due to poor germination and death of plants due to diseases, insect pests and accidents. In some crops, e.g., pulses, plant loss may be one of the most serious problems of the scheme.

Exercise:

1. Prepare a field note book for handling a segregating population.

Practical 10: Study of field techniques for seed production and hybrid seeds production in *Rabi* crops.

Procedure of hybrid seed production of mustard

https://www.youtube.com/watch?v=QpTAu8e0eG0

Principle of hybrid seed production:

Cytoplasmic Genetic Male Sterility (CGMS) system is used for commercial hybrid seed production of mustard. The male sterile line (A line) contains sterile cytoplasm and recessive genes for fertility restoration. This is maintained by a male fertile counterpart (line B) which also contains recessive genes, but has fertile cytoplasm.

For production of hybrid seed male sterile line (A line) is crossed with a fertility restoring line (R line) which has the dominant genes for fertility restoration, but may have either sterile or fertile cytoplasm. The restorer line (R line) should nick well with 'A' line to produce F_1 hybrid seed.

1. **Production of male sterile line ('A'line) Seed:**

- **Isolation Requirements:** Seed fields must be isolated from other brassica field and same line increase fields not conforming to varietal purity requirements of certification at least by 200 meters.
- **Planting Ratio:** The proportion of female line (A line) and male line (B line) should be 4:2. In order to have pollen pressure two-four border rows of B-line all around the field may be grown.
- **Roguing:** The off-type plants distinguishable on the basis of morphological characteristics should be removed before flowering. The seed crop should be sown in wide rows (30 cm) to permit rouging.
- **Harvesting:** The B-line should be harvested just after flowering period is over and prior to harvest of female rows to avoid contamination.

2. **Production of maintainer line (B-line) and restorer line (R-line) seed:**

- The B-line and R-line are self-fertile and may be multiply by open-pollination in an isolated field having isolation distance 200 m.
- **Spacing:** Row to row distance 42-45 cm.

3. **Production of hybrid sunflower seed:**

- **Isolation requirements-** Seed fields must be isolated at least by 200 meters from

the fields of other brassica and same hybrid seed production not conforming to varietal purity requirements of certification.

- **Planting ratio:** The proportion of female parent (A line): male line (R line) should be kept at 3:1. However, the first two border rows on either side may be sown with the male parent seed to suplly enough pollen.

Roguing: The off-type plants distinguishable on the basis of morphological characteristics should be removed before flowering. Remove objectionable weed plant.

Spacing: Row to row: 30cm and plant to plant: 20 cm

Seed rate: A line 1.88 kg per ha.

R line 0.88 kg per ha.

Harvesting: Male lines are harvested before female line when siliqua are mature and attain yellow colour.

Research stations:

A. National:

National Research Centre for Rape Seed and Mustard (NRCRM), Sewar, Bharatpur, Rajasthan

B. State level:

Main Mustard Research Station, Sardarkrushinagar Dantiwada Agri cultural University, Sardarkrushinagar, Gujarat

Achievements:

- Gujarat Mustard-1 **(GM 1)**
- Gujarat Mustard-2 **(GM 2)**
- Gujarat Mustard – 3 **(GM 3)**
- Gujarat Dantiwada Mustard – 4 **(GDM 4)**
- Varuna , Pusa Jay Kisan (Developed by somaclonal variation, IARI, New Delhi)

Procedure of hybrid seed production in sunflower

Principle of hybrid seed production:

- Hybrid sunflower is produced by using cytoplasmic male sterility and genetic fertility restoration system. The male sterile line (A line) contains sterile cytoplasm and recessive genes for fertility restoration. This is maintained by a male fertile

counterpart (line B) which also contains recessive genes, but has fertile cytoplasm.

- For production of hybrid seed male sterile line (A line) is crossed with a fertility restoring line (R line) which has the dominant genes for fertility restoration, but may have either sterile or fertile cytoplasm. The restorer line (R line) should nick well with 'A' line to produce F_1 hybrid seed.

1. **Production of male sterile line ('A'line) Seed:**

- **Isolation Requirements:** Seed fields must be isolated from other sunflower field, same line increase fields not conforming to varietal purity requirements of certification and wild sunflower species at least by 600 meters.
- **Planting Ratio:** The proportion of female line (A line) and male line (B line) should be 3:1. However, the first two border rows on either side may be sown with the male line (B line) seed to ensure enough pollen supply.
- **Seed rate:** A line: 7.5 kg/ha and
  B line: 2.5 kg/ha

- **Roguing:** The male-fertile plants in the female parent lines should be removed each day during the entire flowering period. This is best done in the morning hours before the bees have removed the pollen.
- **Supplementary pollination:** For supplementary pollination (Hand pollination) the palm is first gently rubbed on the male parent flowers and then on the stigmas of the female line to transfer the pollen.
- **Harvesting:** The male parent rows should be harvested prior to harvest of female rows to avoid contamination. No male parent heads should be left intermingled with the female parent rows.

2. **Production of maintainer line (B line) and restorer line (R line) seed:**
   The seed is produced in an isolated field having isolation distance 600 m
3. **Production of hybrid sunflower seed:**

- **Isolation requirements**- Seed fields must be isolated atleast by 400 meters from the fields of other varieties, commercial hybrid of the same variety, fields of same hybrid seed production not conforming to varietal purity requirements of certification.
- **Planting ratio:** The proportion of female parent (A line): male line (R line) should be kept at 3:1. However, the first two border rows on either side may be sown with the male parent seed to supply enough pollen.

- **Seed rate:** A line 7.5 kg per ha.
  - R line 2.5 kg per ha.
- Other practices are the same as described for the A line seed production.

Achievements:

a) **Improved varieties**: Modern, Surya, TNAU SUF 7, GAU-SUF-1, EC-68413,
   EC-68414, EC-68415 and EC-69874
b) **Hybrid varieties:** BSH-1, KBSH-1, KBSH-11

Research stations:

a) **National:** Directorate of Oilseed Research, Hyderabad
b) **State:** Main Oilseeds Research Station, JAU, Amreli, Gujarat.

Exercise:

1. **Explain detail procedure of hybrid seed production in mustard.**
2. **Explain detail procedure of hybrid seed production in sunflower**

Practical 11. Estimation of heterosis, inbreeding depression and heritability.

https://www.youtube.com/watch?v=V_pbiomiMSY&t=175s

Heterosis: The superiority of F1 (hybrid) in one or more characters over its parents

Types:

A. Average heterosis
B. Standard or useful heterosis
C. Heterobelthiosis
D. Luxuriance heterosis

HOW TO ESTIMATE:

A. **Average Heterosis (A. H.) -** When the F_1 is superior than over mid parent.

A.H. = F_1 – MP / MP x 100

Example 1. What is the average heterosis if yield of F1 is 48 qtls/ hec and yield of P1 and P2 respectively 40 and 50 qtls/ hec

Answer:

F1 = 48 qtls/hec

P1 = 40 qtls/hec

P2 = 50 qtls/ hec

MP = P1 + P2 / 2

= 40 + 50 / 2 = 45

A.H. = F1 – MP / MP x 100

= 48 – 45 / 45 x 100 = 6.66%

B. **Standard or useful Heterosis (S. H.):** when the F_1 is superior than standard check variety (SCV)

S.H. = F1 – SCV/ SCV x 100

Example 2: What is the standard heterosis if yield of F_1 is 50 qtls/hec and yield of standard check variety 48 qtls/ hec

Answer:

F1 = 50 qtls/hec

SCV = 48 qtls/hec

S. H. = F1- SCV / SCV x 100

= 50 – 48 / 48 x 100 = 4.16%

C. **Heterobelthiosis H(BP):** when the F_1 is superior over than better parent.

$$\mathbf{H\ (BP) = F1 - BP\ /\ BP\ x\ 100}$$

Example 3. What is the heterobelthiosis if yield of F_1 is 50qtls/ hec and yield of P1 and P2 respectively 40 and 48 qtls/hec

Answer:

F_1 = 50qtls /hec

P1 = 40qtls/hec

P2 = 48 qtls/hec

H (BP) = F_1 – BP / BP x 100

= 50-48 / 48 x 100 = 4.16%

Inbreeding depression: when loss or decrease in vigour of F_1 (hybrid) due to inbreeding.

$$\mathbf{I.\ D. = F_1 - F_2\ /\ F_1\ x\ 100}$$

Inbreeding depression is reduced fitness in a given population as a result of breeding of related individuals. Breeding between closely related individuals, called inbreeding, results in more recessive deleterious traits manifesting themselves. The more closely related the breeding pair is, the more homozygous deleterious genes the offspring may have, resulting the very unfit individuals. Another mechanism responsible is overdominance of heterozygous alleles leading to a reduction of fitness of a population with many homozygous genotype, even if they are not deleterious. Currently it is not known which of the two mechanism is more important. In general, populations with more genetic variation do not suffer from inbreeding depression. Inbreeding depression is often the result of a population bottleneck. Inbreeding depression seems to be present in most groups of organisms, but is perhaps most important in hermaphroditic species, most prominently in plants. The majority of plants are hermaphroditic and thus capable of the most severe degree of inbreeding.

Calculation: The inbreeding is computed as a percentage of chances for two alleles to be identical by descent. This percentage is called "inbreeding coefficient". There are several methods to compute this percentage, the two main ways are the path method and the tabular method.

Typical inbreeding percentage are as follows:

- Father / daughter – mother / son –brother / sister – 25%

- Half –brother / half- sister – 12.5%
- Uncle / niece – aunt / nephew – 12.5%
- Cousin – 6.25%

An inbreeding calculation may be used to determine the general genetic distance among relatives by multiplying by 2, because any progeny would have a 1 in 2 risk of actually inheriting the identical alleles from both parents. For instance, the parent / child or sibling / sibling have 50% identical genetics.

NOTE: For siblings, the degree of genetic relationship is not an automatic 50% (as it is with parent and their children), but a range from 100% at one extreme – as in the case of identical twins (who obviously could not mate as they are the same sex) – to an exceedingly unlikely 0%. Siblings share an average of 50% of their genes, but unlike the 50% ratio between parents and children, the actual ratio between siblings in any given case can vary widely.

Example 4. What is the inbreeding depression if yield of F_1 is 50 qtls / hec and yield of F_2 40 qtls/hec

F_1 = 50 qtls/hec

F_2 = 40 qtls/hec

I.D. = F_1 - F_2 / F_1 x 100

= 50-40 / 50 x 100 = 20%

Exercise:

In maize, 15 hybrids along with 8 parents were evaluated for heterosis on seed yield. The genotypes were raised in RBD with three replications. Calculate the different types of Heterosis and interpret the result.

Sl no.	Genotypes	RI	RII	RIII
1.	P1	17.0	17.2	17.1
2.	P2	15.0	15.6	15.6
3.	P3	15.3	15.0	15.0
4.	P4	14.7	14.7	14.4
5.	P5	19.8	19.4	19.3
6.	P6	22.2	22.3	22.7
7.	P7	20.1	19.9	20.3
8.	P8	26.8	27.2	26.7
9.	P1 x P6	23.2	23.5	23.5
10.	P2 x P6	22.1	22.1	22.9
11.	P3 x P6	21.3	21.1	19.8

12.	**P4 x P6**	**19.1**	**19.0**	**18.6**
13.	**P5 x P6**	**24.0**	**24.6**	**24.0**
14.	**P1 x P7**	**22.6**	**23.2**	**22.9**
15.	**P2 x P7**	**22.8**	**23.2**	**22.7**
16.	**P3 x P7**	**19.6**	**19.7**	**20.1**
17.	**P4 x P7**	**19.9**	**20.0**	**19.5**
18.	**P5 x P7**	**15.4**	**16.0**	**16.3**
19.	**P1 x P8**	**20.1**	**20.6**	**20.5**
20.	**P2 xP8**	**16.0**	**16.5**	**16.4**
21.	**P3 x P8**	**20.6**	**20.9**	**20.6**
22.	**P4 x P8**	**17.3**	**17.9**	**17.9**
23.	**P5 x P8**	**20.7**	**28.0**	**27.9**
24.	**SC**	**17.6**	**14.8**	**15.0**

Practical 12: Layout of field experiments.

https://www.youtube.com/watch?v=JY6NCfiTReE

The basic objective of plant breeding is the ultimate crop improvement. It results in development of high yielding varieties hybrids etc., over the existing cultivars and so on. The performances of the new varieties are confirmed from the results obtained from the field experiments. To be explained scientifically the field experiments are laid out following certain rules and the data thus collected are analysed statistically. The steps involved in this process are explained here under.

Any designing of experiments involves three major steps.

1. Selection of experimental units

The objects on which the treatments are applied is known as experimental units. Eg. Plots in the field, plant, etc.,

2. Fixing of treatments

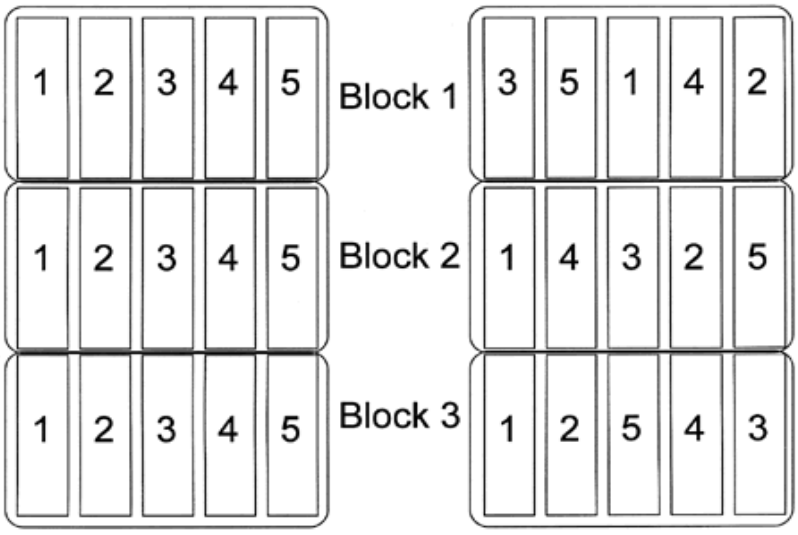
The objects of comparison are known as treatments. Eg. Varieties, spacing etc.,

3. Arrangement of treatments in the experimental Units

It comprises of three basic principles of design

Replication: repetition of treatments

Randomization: unbiased allocation of treatments to the experimental units



Test plots on the left are not randomized. Plots on the right are randomized. The numbers (1-5) represent the five treatments in this test

Local control: minimizing the effect of heterogeneity of the experimental units

The objective of replication, randomization and local control is to minimize the Experimental Error (EE). EE is nothing but differences in the responses from the experimental unit to experimental unit under similar environments. Apart from these, EE can be reduced further by proper selection of the experimental units and choosing of most appropriate experimental design for a given number of treatment.

Types of basic experimental designs

1. Completely Randomized Design (CRD)
2. Randomized Block Design (RBD)
3. Latin Square Design (LSD)

Among these, RBD is the widely used design.

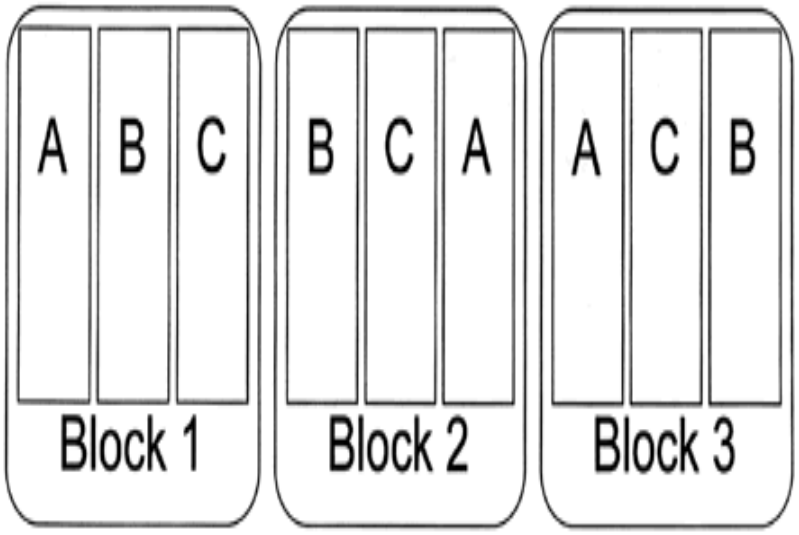
Laying Out of RBD

A. The experimental material (field) is divided first into blocks consisting of homogenous (uniform) experimental units. Each block is divided into number of treatments equal to the total number of treatments.

B. Randomization should be taken within each block and the treatments are applied following the random number table.

C. Collection and analysis of data: After the collection of data from the individual experimental unit (treatments) ANOVA (Analysis of Variance) table is formed.

The significance of the ANOVA table is that it indicates the sources of variation exhibited by the treatments, the magnitude of variation derived from different sources and their worthiness (significant/ non-significant).



An easy way to arrange blocks is to put them side by side across the field. Letters represent different treatments.

D. Computation of Critical Difference (CD)

Critical Difference is the difference between the treatment means, which places the treatments statistically as well as significantly apart. Otherwise if the difference of two treatments mean is less than CD it can be concluded both the treatments are on par.

RT: Row trial

Row trial is generally conducted in F3 and F4, when the seeds are not sufficient for replication with individual plant progeny rows. Each row consists of about 20 or more plants. Individual plants with desirable characteristics are selected from superior progeny rows. Pest, Disease and lodging susceptible progenies with undesirable characteristics are eliminated.

RRT – Replicated Row Trial

It is generally conducted from F3 generation onwards. Depending on availability of seeds, 3-4 more rows are grown for each progeny to facilitate comparison among progenies adopting suitable replications. Families, which have become reasonably homozygous may be harvested in bulk. From those families showing segregation, single plants are selected for characters under study. The breeder has to visually assess the yielding potential of progenies and reject the inferior ones in the field and the yield potential has to be assessed in the laboratory for confirmation.

PYT – Preliminary Yield Trial or Initial Yield Evaluation Trial (IYET)

It is conducted from F5 generation onwards. Preliminary yield trial with three or more replications are conduct to evaluated the comparative performance of the culture and to identity the superior cultures among them. The cultures are evaluated for plant height, lodging, pest and disease resistance, flowering time, duration and yield, etc., Quality tests may also be carried out. Standard commercial varieties must be included as checks for comparison. Ten to fifteen outstanding cultures, if superior to checks, would be advanced to the Advanced yield trails.

AYT – Advanced Yield Trial

Advanced Yield Trial is conducted from F8 generation onwards. The superior cultures identified from Preliminary Yield Trial are tested in Replicated Yield Trial. In this trial, the cultures are evaluated for yield, pest, disease and lodging resistance, duration, quality, etc.

MLT - Multi Location Trial

Multi location trial is conducted from F13 onwards for 3 years by the Research Station Scientists. Multi Location Trial are useful for suitability studies i.e. whether a particular culture is able to perform well in all the locations or not and whether the particular culture out yields all the other cultures developed by research stations and the check variety evaluated

simultaneously. Based on the evaluation, superior and stable performing culture will be promoted to ART.

ART – Adaptive Research Trial

It is conducted after MLT for 3 years by the Department of Agriculture. Nearly 3-4 cultures are tested and based on the performance of 3 Years in the farmer's field, the best culture over the check may be proposed to SVRC (State Variety Release Committee) for releasing.

If the SVRC finds that the cultivar is suitable for any particular area or throughout the state, then the variety is released and is notified by the State Department of Agriculture.

Exercise:

1. Draw a layout of the following data

Trait: yield / plant

Varieties: 50

Replication: 3

Practical 13: Study of quality characters, study of donor parents for different characters.

https://www.youtube.com/watch?v=F-UN3Ny4ql0

COMMON NAME	SCIENTIFIC NAME	CHARACTER NO.	QUALITY CHARACTERS
Wheat	*Triticum aestivum*	2n=14 2n=28 2n=24	• Glutamine content • Dough • Flour quality • Water holding capacity of humus
Sunflower	*Helianth as annus*	2n=34	• High oil content (46-54%)
Mustard	*Brassica compestris*	2n=20	• High oil content (40-45%) • Protein content (30-41%)
Chili	*Capsicum annum*	2n=24	• Fruit length • Ascorbic acid content • Capsacino content
Chrysanthemum	*Chrysanthemum monifolium*	2n=13	• Flowering quality, average flower weight, flower diameter
Gerbera	*Gerbera*	2n=50	• Colour of flower leaf and leaf area no. of flowers/plant shelf life of flowers buckers production capacity
Mango	*Mangifera indica*	2n=40	• Self-life of fruit high ascorbic acid %
Guava	*Psidium guajava*	2n=22	• Colour, flower, texture, taste of fruit
Rose	*Rosa sp*	2n=14	• Large flower bud maximum bud diameter, no. of petals/flowers
Papaya	*Carica papaya*	2n=18	• TSS, pH, ascorbic acid
Banana	*Musa sp*	2n=22	• Fresh appearance of fruit self like, shape of fruit at pedicel
Tomato	*Solanum lycopersicum Lycopersicum esculantum*	2n=24	• Fruit uniformity

Exercise:

1. Mention the different quality characters of *Rabi* crops under your study.

Practical 14: Visit to seed production plots.

VISIT TO VARIETAL / HYBRID SEED PRODUCTION PLOT

Date:

Q.1 Where did you visit a seed production plot?

Q.2 Name the crop in which seed production was taken up by the farmer/organizer.

Q.3 What are the criteria for selection of a site for production plot?

Q.4 Write the Name of variety/hybrid and its parentage details.

Q.5 What was the isolation distance and planting ratio kept?

Q.6 What were the important stages of rouging in this seed production plot?

Q.7 Write the following agronomical details of seed production plot?

1. Date of sowing:

2. Spacing: a) Row to row:

b) Plant to plant:

3. Fertilizer: a) Basal:

b) Top dressing:

4. Number of irrigation:

5. Number of weeding

Q.8 What was the expected yield of hybrid/ variety seed production?

Practical 15: Visit to AICRP plots