The Neotia University

A practical manual on Fundamentals of Plant Breeding

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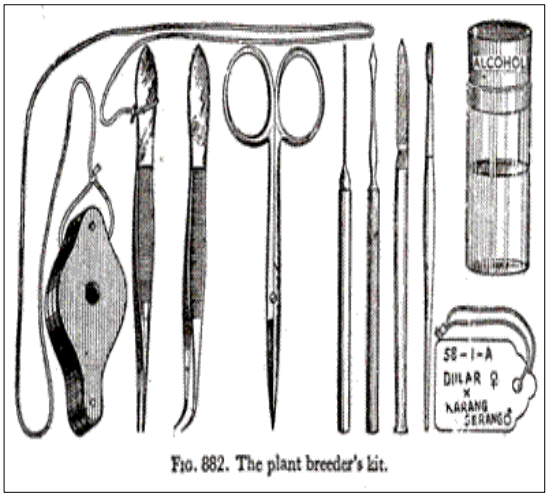

FIG. 882. The plant breeder's kit.

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Laboratory Manual

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Practical 1. Plant Breeder's kit

https://www.youtube.com/watch?v=y_xOe_rnIS8

A breeder requires the following tools for controlled selfing, artificial pollination and for field observation.

Sl. No.	Items	Purpose
1.	Magnifying lens	To observe small flowers, stigmatic surface, dehiscence of anthers etc.
2.	Pair of forceps	Fine two pointed forceps are required for emasculation.
3.	Pair of scissors	Fine pointed straight or curved scissors required to remove unwanted buds, awn etc.
4.	Needles	Required to open small buds and separating the floral parts
5.	U-pin, wire rings, spool of thread	Used to set the paper bag on flowers / inflorescence
6.	Brushes	Camel hair brushes of size 3 or 4 for collection of pollen and transfer to stigma.
7.	Petri dish	Required for collecting pollen grains
8.	Bags	Butter paper bag, muslin cloth, and paper bags of different sizes for different crop.
9.	Alcohol or Methylated spirit	A small vial of alcohol or methylated spirit is required to sterilize forceps, scissors, needles, brushes etc.
10.	Tags	Paper, cardboard or aluminium tags are required for labelling the units in the field. In the case of paper or cardboard tags, they have to be dipped in wax after labelling and tags are tied in bamboo stick
11.	Meter scale	Required for plant measurement in the field
12.	Field note books pen / pencils	Field note books are required to note down daily observation in the field, regarding germination, flowering, morphological description

Exercise 1. Draw different component of breeder's kit.

Practical 2. Study of germplasm of various crops

https://www.youtube.com/watch?v=zQuhmJ95_XM

Principle:

Germplasm is living tissue from which new plants can be grown. It can be a seed or another plant part – a leaf, a piece of stem, pollen or even just a few cells that can be turned into a whole plant. Various institutes with different objectives are engaged in plant and/or germplasm collecting activities. The collecting of plant genetic resources primarily aims at tapping germplasm variability in different agri-horticultural (crop) plants, their wild relatives and related species. The germplasm so collected reveals the nature and extent of variability in different species, within species, cultigens, etc. as also their agro-ecological / phyto-geographical distribution. Knowledge of agro-ecology, crops and their distribution and harvesting time in areas of survey, local contacts, equipment required, transport arrangements and routes to be followed, distances involved, places of halt / camping sites available, transport of material, besides team-composition etc. is to be acquired before setting out on a collecting expedition. Of equal importance is to acquire knowledge on diversity in crop plants vis-a-vis its distribution to tap target areas and / or target species and the variability contained thereof.

Germplasm collecting strategies

A. For seed collections (cultivated and wild species)

1. Collect from (30- 100) individuals per site (50 seeds of each as one sample or less, if necessary, at random. One inflorescence per plant is generally suitable.
2. Sample as many sites as possible according to availability of time.
3. Choose sampling sites over as broad an environmental range as possible. This should capture all alleles with frequency of 5 percent or more in the population.
4. Change tactics, where necessary, for wild species, that is, where individuals are scattered, you may need to consider that a population for sampling spreads over several square kilometres.
5. If considerable morphological variation is present in a population, make separate samples of each type.
6. Take whole inflorescences, as well as seeds, where necessary, as vouchers.
7. Make herbarium specimens, where possible.
8. Take photographs.
9. Write meticulous field notes.

B. For collecting wild vegetatively propagated species

Collect just a single propagule from each of 10-15 individuals as a bulk sample (less if organs are very large, more if smaller, from area of about 100 x 100 m).

Germplasm cataloguing, Data storage and Retrieval

- Each germplasm accession is given an accession number. This number is pre fixed in India, with either IC (Indigenous collection), EC (exotic collection) or IW (Indigenous wild).
- Information on the species and variety names, place of origin, adaptation and on its various feature or descriptors is also recorded in the germplasm maintenance records.
- Catalogues of the germplasm collection for various crops are published by the gene banks.
- The amount of data recorded during evaluation is huge. Its compilation, storage and retrieval are now done using special computer programmes.

Gene bank for various crops in India

1.	Central Institute of Cotton Research, Nagpur	--	Cotton
2.	Central Plantation crops Research Institute, Kasaragod	--	Plantation Crop
3.	Central Potato Research Institute, Simla	--	Potato
4.	Central Tobacco Research Institute, Rajahmundry	--	Tobacco
5.	Central Tuber crop Research Institute, Thiruvananthapuram	--	Tuber crops other than Potato
6.	National Rice Research Institute, Cuttack	--	Rice
7.	Directorate of Oilseeds Research, Hyderabad	--	Oilseeds
8.	Directorate of Wheat Research, Karnal	--	Wheat
9.	Indian Agricultural Research Institute, New Delhi	--	Maize
10.	Indian, Grassland and Fodder Research Institute, Jhansi	--	Forage and fodder crops
11.	National Research Centre for Sorghum, Hyderabad.	--	Sorghum

Germplasm Conservation

Conservation refers to protection of genetic diversity of crop plants from genetic erosion. There are two important methods of germplasm conservation or preservation. i) in-situ conservation, ii) ex-situ conservation.

These are described below.

i) In-situ conservation

Conservation of germplasm under natural conditions is referred to as in situ conservation. This is achieved by protecting the area form – human interference, such an area is often called natural park, biosphere reserve or gene sanctuary. NBPGR, New Delhi, established gene sanctuaries in Meghalaya for citrus, north Eastern regions for Musa, citrus, Oryza and *Saccharum.* Gene sanctuaries offer the following advantage.

Merits: In this method of conservation, the wild species and the complete natural and semi natural ecosystems are preserved together.

Demerits:

1. Each protected are will cover only very small portion of total diversity of crop species, hence several areas will have to be conserved for single species.
2. The management of such areas also poses several problems.
3. This is a costly method of germplasm conservation.

ii) Ex-situ conservation

It refers to preservation of germplasm in gene banks. This is the most practical method of germplasm conservation. this method has following advantages.

1. It is possible to preserve entire genetic diversity of a crop species at one place
2. Handling of germplasm is easy.
3. This is the cheap method of germplasms conservation

This type of conservation can be achieved in the following 5 ways.

1. Seed banks:

Germplasm is stored as seeds of various genotypes. Seed conservation is quite easy, relatively safe and minimum space. Seeds are classified, on the basis of their storability into two major groups.

1) Orthodox 2) Recalcitrant

Orthodox seeds: Seeds which can be dried to low moisture content and stored at low temperature without losing their viability for long periods of time is known as orthodox seeds. E.g. Seeds of corn, wheat, rice, carrot, papaya, pepper, chickpea, cotton, sunflower.

Recalcitrant: Seeds which show very drastic loss in viability with a decrease in moisture content below 12 to 13% are known as recalcitrant seeds. E.g. citrus, cocoa, coffee, rubber, oil palm, mango, jackfruit etc.

Seed storage:

Based on duration of storage, seed bank collects are classified into three groups. 1) Base collection 2) Active collection and 3) Working collection

Base collection: Seeds can be conserved under long term (50 to 100 years), at about -20°C with 5% moisture content.

Active collections: Seeds are stored at 0°C temperature and the seed moisture is between 5 and 8%. The storage is for medium duration, i.e., 10-15 years. These collections are used for evaluation, multiplication and distribution of the accessions.

Working collections: Seeds are stored for 3-5 years at 5-10°C and usually content about 10% moisture. Such materials are regularly used in crop improvement programmes.

2. Plant Bank:

(Field or plant bank) is an orchard or a field in which accessions vegetatively propagated crops are grown and maintained.

Limitations:

1. Require large areas
2. Expensive to establish and maintain.
3. Prone to damage from disease and insect attacks.
4. Man-made
5. Natural disasters
6. Human error handling

3. Shoot tip banks

Germplasm is conserved as slow growth cultures of shoot-tips and node segments. Consevation of genetic stocks by meristem cultures has several advantages as given below.

1. Each genotype can be conserved indefinitely free from virus or other pathogens.
2. it is advantageous for vegetatively propagated crops like potato, sweet potato, cassava etc., because seed production in these crops is poor.
3. Vegetatively propagated material can be saved from natural disasters or pathogen attack.

4. Long regeneration cycle can be envisaged from meristem cultures.

5. Regeneration of meristems is extremely easy.

6. Plant species having recalcitrant seeds can be easily conserved by meristem culture.

4. Cell and organ banks:

A germplasm collection based on cryopreservation (at - 196°C in liquid nitrogen) embryo culture, somatic / zygotic embryos they be called cell and organ bank.

5. DNA banks

In these banks, DNA segments from the genomes of germplasm

Germplasm evaluation

Evaluation refers to screening of germplasms in respect of morphological, genetical, biochemical, physiological, pathological and entomological attributes. Evaluation of germplasm essential from following angels.

1. To identify gene sources for resistance to biotic and abiotic sources, earliness and quality characters.

2. To classify the germplasm into various groups.

3. To get a clear picture about the significance of individual germplasm line.

National Bureau of Plant Genetic Resources (NBPGR)

NBPGR establishment in 1976 is the nodal organisation in India for planning, conducting, promoting, coordinating and lending all activities concerning plant.

List of important International Institutes

Name	Institute	Activities
IRRI	International Rice Research Institute, Los Banos, Philippines	Tropical rice, Rice collection: 42, 000
CIMMYT	Centre International de-Mejoramients de maize Y Trigo, El Baton, Mexico	Maize and wheat (Triticale, barley, sorghum) Maize collection - 8000
CIAT	Center International de-agricultural Tropical Palmira, Columbia	Cassava and beans, (also maize and rice) in collaboration with CIMMYT and IRRI
IITA	International Institute of Tropical Agriculture, Ibadan, Nigeria	Grain legumes, roots and tubers farming systems.
CIP	Centre International de-papa-Lima, Peru	Potatoes

ICRISAT	International Crops Research Institute for Semi-arid Tropics, Hyderabad	Sorghum, Groundnut, Pigeon pea, Bengal gram, Red gram.
WARDA	West African Rice Development Association, Monrovia, Liberia	Regional cooperative Rice Research in collaboration with IITA and IRRI.
IPGRI	International Plant Genetic Research Institute, Rome, Italy	Genetic conservation

Exercise:

1. Visit the germplasm evaluation and maintenance units of different crops
2. Differentiate between the following
    a. Base, Active and working collection

Practical 3. Study of floral structure of self-pollinated

https://www.youtube.com/watch?v=8HlaxqWk0KU

Method of studying the floral structure of a plant

A. INFLORESCENCE: A cluster of flowers or arrangement of flowers on floral axis.

Terms

I. **RACEMOSE:** An inflorescence where the main axis dose not terminate in a flower, but it continuous to grow and gives off flowers laterally in acropetal succession (Fig. 1).

- **a) Raceme:** A simple, elongated, indeterminate inflorescence with stalked flower. E.g. Radish, Mustard etc.
- **b) Panicle:** When axis of raceme is branched, it is called a panicle. E.g rice, wheat etc.
- **c) Spike:** Usually unbranched, elongated, simple, indeterminate inflorescence whose flowers are sessile, e.g.
- **d) Spikelet:** the unit of the compound inflorescence of the grasses, composed of a cluster of one or more flowers and their associated bracts, e.g: grasses.
- **e) Spadix:** A thick or flashy spike subtended or surrounded by a spathe, e.g: Maize.
- **f) Corymb:** Indeterminate inflorescence with shortened main axis, in which the lower flowers have much longer pedicels than the upper so that flowers are brought more or less to the same level. **E.g:**
- **g) Umbel:** An inflorescence in which the flower stalks of more or less equal length, arise from the same point, like the ribs of an umbrella at the base of flower stalks, there is whorl of bracts forming an involucre. E.g.
- **h) Compound umbel:** An umbel with branched axis and the branches bearing the flowers. These are known as umbellule. E.g : Coriander
- **i) Capitate:** When a large number of sessile flowers arise from a suppressed axis forming a globose structure as in Acacia, Mimosa. It differs from capitulum in the absence of a receptacles.
- **j) Capitulium:** A dense inflorescence comprising an aggregation of usually sessile flowers arranged on a convex receptacle formed by the axis, and having one or more wholrs of bracts forming involucre, e.g : composite family.

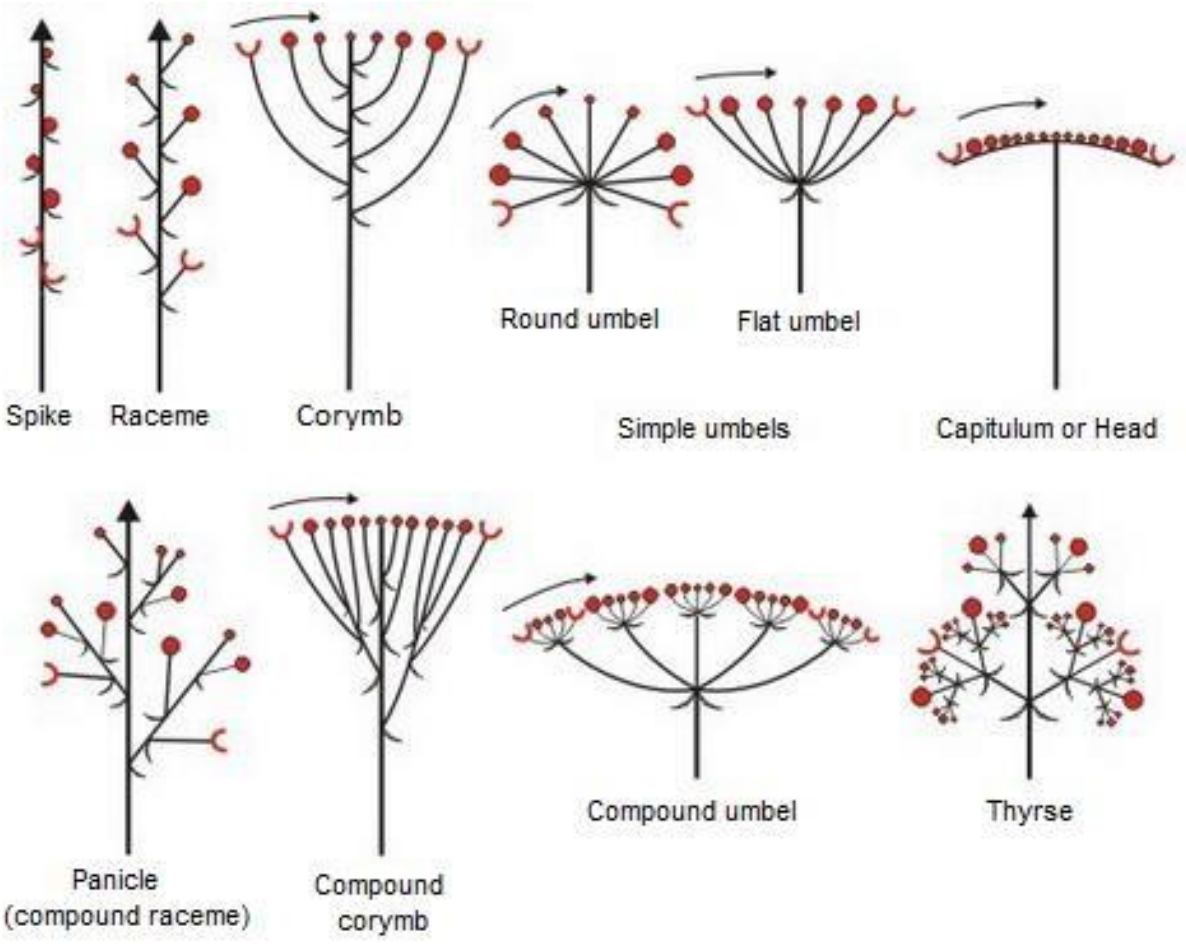


Fig. 1. Types of racemose inflorescence

II. CYMOSE: An inflorescence where the growth of the main axis is soon checked by the development of a flower its apex and the lateral axis which develops below the terminal flower also ends in a flower, thus its growth is also checked (Fig. 2).

a) Uniparous: The main axis ending in a flower producing only one lateral branch at a time ending in a flower. Also known as scorpioid and helicoid**.**

b) **Biparous:** A determinate inflorescence in which the main axis ends in a flower after producing to daughter axes of flowers. E.g: Ixora

c) Multiparous: A determinate inflorescence in which the main axis end in a flower after producing a number of daughter axes or flowers around. This inflorescence looks like an umbel but can be distinguished from umbel by the opening of the middle flower first. E.g: Calotropis.

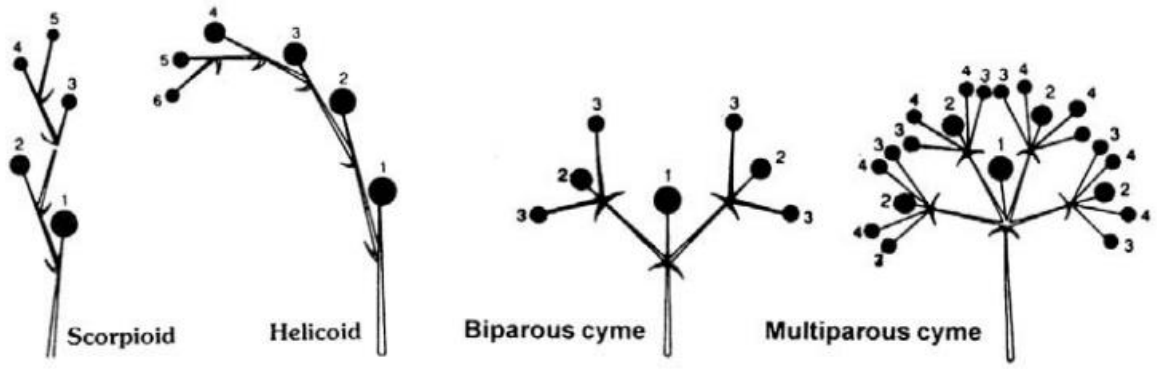


Fig. 2. Types of cymose inflorescence

III. **SOLITARY FLOWER:** Borne singly or alone.

a) **Solitary terminal:** Borne singly at the apex, (Fig. 3) e.g. Poppy

b) **Solitary axillary:** Borne singly in the axil of a leaf (Fig 4)-. E.g.: Cucurbita, china rose

Fig. 3. Solitary terminal

Fig. 4. Solitary axillary

B. FLOWER: Modified shoot, meant essentially for reproduction of the plant.

I. Bract and Bracteoles

Terms

a) Bract: A modified, usually reduced leaf like structure at the base of the flower.

b) Bracteoles: Bract occurring on secondary axis i.e. pedicel of the flower.

c) Ebracteate: Without bracts. E.g. solanum

d) Bracteate: with bract. E.g. Adhatoda

e) **Bracteolate:** with bracteoles. E.g. Adhatoda

f) **Epicalyx:** Whorl of bracteoles developing the base of the calyx. E.g: China rose

II. Attachment of the flower

Terms

a) **Pedicel:** Stalk of an individual flower

b) **Peduncle:** The stalk of an inflorescence.

III. PRESENCE OF FLORAL WHOLRS

Terms

a) **Complete:** A flower with four whorls of floral parts; pistil, stamens, sepals and petals. E.g: Solanum.

b) **Incomplete:** A flower lacking one or more of the four kinds of floral sepals, petals, stamens or pistils. E.g. Euphorbia

IV. SYMMETRY

Terms

a) **Actinomorphic:** (Regular). Applied to a flower in which the parts of each whorl are similar in shape. The flowers can be divided into two equal halves along more than one median longitudinal plane: E.g: Okra

b) **Zygomorphic:** Terms applied to a flower in which the members of some or all of the floral whorls are unequal. Most irregular flowers can be divided longitudinally into two qual halves in only one vertical plane. E.g. pea

V. PRESENCE OF REPRODUCTIVE ORGANS

Terms

a) **Hermaphrodite:** (Bisexual). A flower in which both stamens and pistils are present. E.g: Cina rose.

b) **Unisexual:** A flower having only one sex. E.g cucurbits

c) **Staminate:** A unisexual flower with stamens

d) **Pistillate:** A unisexual flower with pistl.

VI. POSITION OF FLORAL ORGANS ON THALAMUS

a) **Hypogynous:** A flower in which the ovary is superior and all other floral organs are situated below its level. E.g: Citrus

b) **Epigynous:** Used for a flower when the ovary is inferior and other floral organs arise above it. E.g Coriandrum.

C. CALYX: The outer or first whorl of a flower consisting sepals

Sepal. One of the separate parts of a calyx, usually green and foliaceous.

I. No. of sepals: Mention the number of sepals. E.g. 4 sepals

II. Cohesion:

a) **Polysepalous:** when the sepals are free.

b) Gamosepalous: when the sepals are fused.

III. Aestivation. The arrangement of floral parts (sepals in this case) in bud Fig. 5.

a) Valvate: Sepals meeting by the edges without overlapping, e.g. Solanum

b) **Twisted:** One margin of the sepal overlaps that of the next one, and the other margin is overlapped by the third one.

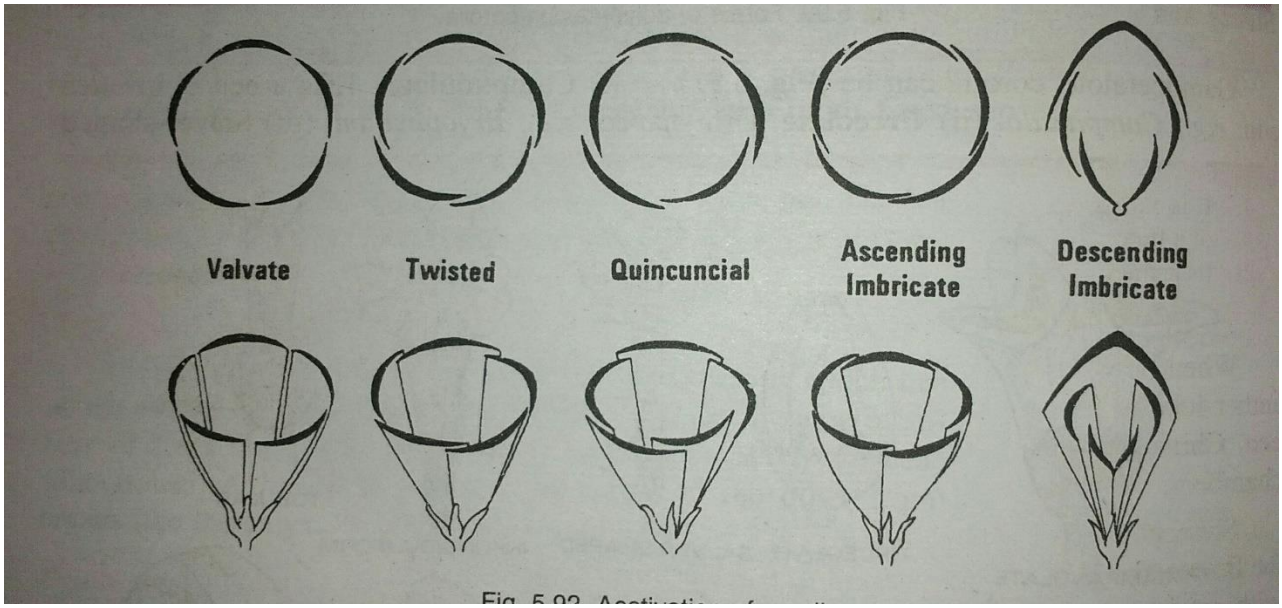


Fig. 5. Types of aestivation

IV. Duration of calyx.

a) Deciduous: Falling off along with the petals just after fertilization. E.g: Mustard.

b) Persistence: Remaining attached in the fruit also. E.g: solanum. Datura.

D. COROLLA: Second whorl of flower made of petals.

Petal. One of the separate parts of corolla usually coloured and more less showy.

I. Number of petals: Mention the number of petals. E.g : 4 petals

II. Cohesion

a) Polypetalous: When the petals are free. E.g: mustard

b) Gamopetalous: When the petals are united.

III. Aestivation

a) Valvate: Petals meeting by the edges without overlapping. E.g: Solanum

b) Twisted: One margin of the petal overlaps that of the next one, and the other margin is overlapped by the third one. E.g.: China rose

c) **Vexillary:** out of the five petals the posterior one is the largest and covers the two lateral petals and the latter in their turn overlap the two interior and smallest petals, characteristics of papilionaceae.

E. PERIANTH: Sometimes calyx and corolla are not distinguishable from one another and the outer whorl is thus called perianth.

Tepal. One of the separate parts of perianth.

F. ANDROECIUM: The male whorl of the flower. Made of stamens.

Stamen: An individual part of an androecium that produces pollen grains usually composed of anther, connective and filament.

I. Number of stamens

Mention the number of stamens e.g. 5 stamens (Fig. 6).

Terms

a) **Polyandrous:** Said of an androecium whose stamens are free. (anthers as well as filament).

b) **Monadelphous:** Stamens united in one group by connation of their filament. (Anthers begins free) e.g: China rose

c) **Diadelphous:** Stamens united in two bundles by connation of their filament (anthers begin free) e.g. pea

d) **Polyadelphous:** Stamens united in many bundles by connation of their filaments. (anthers begin free)

e) **Synandrous:** Stamens united throughout their whole length by both the filaments and the anthers. E.g.: Cucurbita

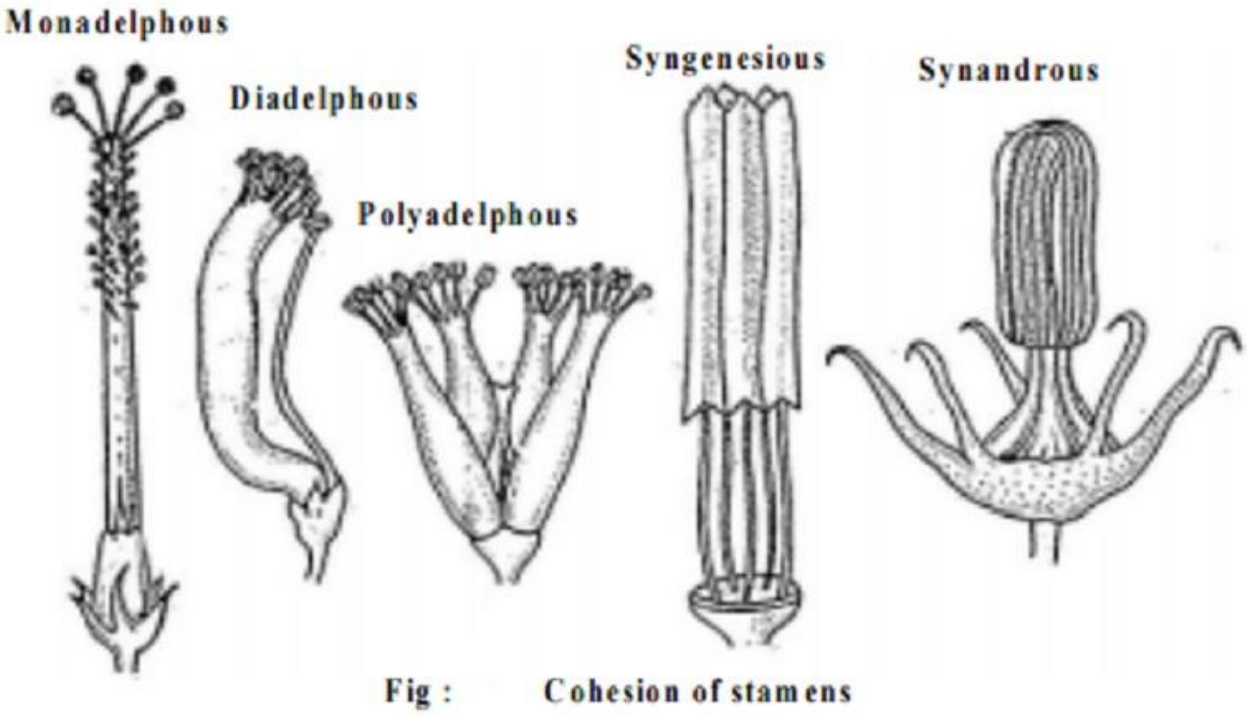


Fig. 6. Types of stamens

II. Length of filaments:

Terms

a) Didynamous: Out of the four stamens two are long and two shorts. E.g: Ocimum

b) **Tetradynamous:** Out of six stamens four inner are long and two outer short. E.g mustard

III. Attachment of filament to anther

Terms

a) Basifixed. Filament attached to the base of the anther (Fig. 7.) E.g. Mustard

b) Dorsifixed: Filament attached to the back of the anther (Fig. 7.). E.g: Citrus, Bauhnia.

c) Versatile: Filament attached to the back of the anther at a point only, so that the latter can swing freely (Fig. 7.) E.g: Grasses.

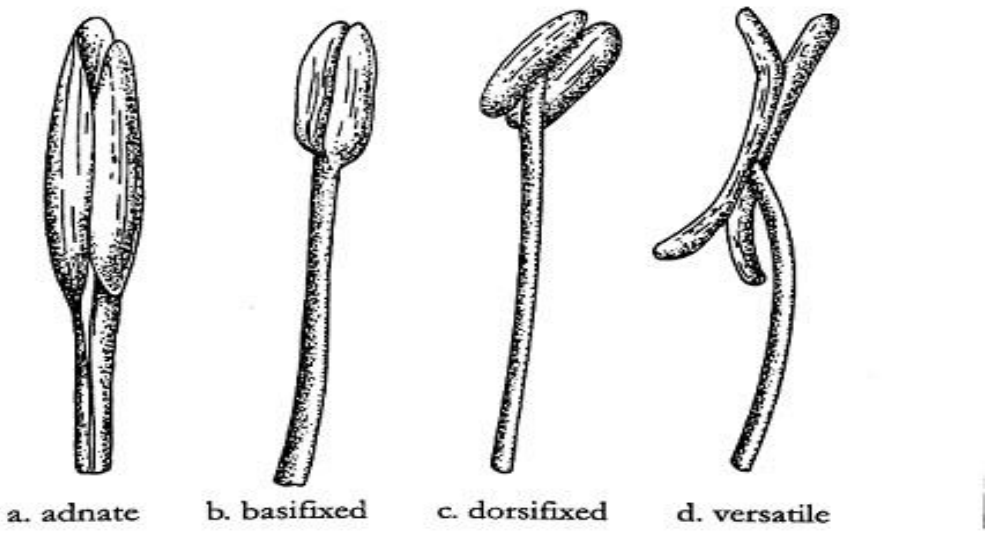


Fig. 7. Types of filament

G. GYNOECIUM: The female whorl composed of one or more carpels.

Carpels: A leaf like organ bearing ovules along the margins, the unit structure of a compound pistil.

I. Number of carpels

a) Simple /monocarpellary gynoecium: When the gynoecium is made up of only one carpel, e.g. pea

b) Compound gynoecium: When the gynoecium is made up of two or more carpels.

II. Cohesion of carpels

a) Apocarpous: A pistil of two or more carpels which are free.

b) Syncarpous: A pistil of two or more carpels which are fused.

III. Position of ovary:

a) **Superior:** When the ovary occupies highest position on thalamus and stamens, petals and sepals are successively inserted below (Fig 8). E,g: Citrus

b) **Semi inferior:** when the thalamus grows around the ovary to form a cup and bears sepals, petals and stamens on the rim of the cup (Fig. 8) e.g Rose

c) Inferior: When the thalamus completely covers the ovary getting fused with it and bears sepals, petals and stamens on the top of the ovary. (Fig 8) e.g. Cucurbita.

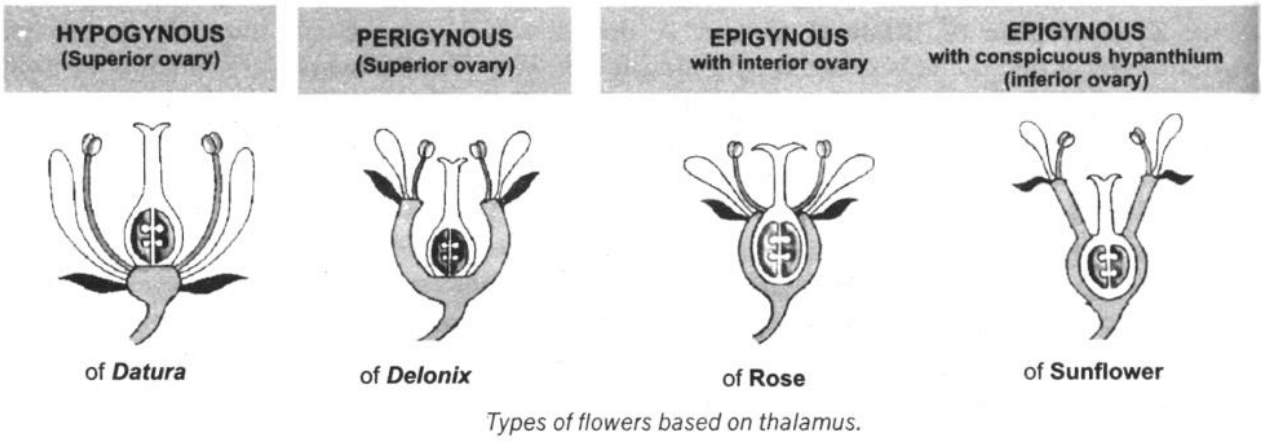


Fig. 8. Different position of ovary

IV. Number of locules

a) Unilocular: Which one chamber

b) Bilocular: Which two chambers

c) **Trilocular:** With three chambers

d) **Tetralocular:** With four chambers

e) **Pentalocular:** With five chambers

f) **Multilocular:** With many chambers

V. Placentation: Arrangement of placentae and ovules in the ovary.

a) **Placenta:** The region or area of the ovary to which ovule or ovules are attached.

b) **Ovule:** The body which encloses embryo sac or female gametophyte and becomes seed after fertilization.

VI. Style: More or less elongated part of gynoecium between the ovary and the stigma.

VII. Stigma: Terminal part of gynoecium that receives pollen grains.

H. FLORAL FORMULA

Once the description of the plant is completed, major characters of a flower are written in a special way where a few signs and letters are used. This formula is useful in knowing major characters of a flower at one glace. In this method characters of bracts, symmetry, sex, calyx, corolla (perianth), androecium and gynoecium are denoted in this order. Some of the commonly used denotation are given below. Choose those or modify according to the need amongst the following few.

Bracts and Epicalyx

- ✓ Bracteate — Br
- ✓ Ebracteate — Ebr
- ✓ Bracteolate — Brl
- ✓ Epicalyx — E

Symmetry

- ✓ Actinomorphic — ⊕
- ✓ Zygomorphic — %

Sex

- ✓ Staminate flowers — ♂
- ✓ Pistillate flowers — ♀
- ✓ Bisexual (Hermaphrodite) — ⚥

Calyx

- ✓ Calyx — K
- ✓ Four free sepals (polysepalous) — K_4
- ✓ Four fused sepals (gamosepalous) — $K_{(4)}$

Corolla

- ✓ Corolla — C
- ✓ Four free petals (polypetalous) — C_4
- ✓ Four fused petals (gamopetalous) — $C_{(4)}$

Perianth

- ✓ Perianth — P
- ✓ Six free tepals (polytepalous) — P_6
- ✓ Six fused petals — $P_{(6)}$
- ✓ Six tepals in two whorls of three each — P_{3+3}

Androecium

- ✓ Androecium — A
- ✓ Five free stamen (polydelphos) — A_5
- ✓ Five fused stamens (monadelphous) — $A_{(5)}$
- ✓ Ten stamens in two whorls of the five each — A_{5+5}
- ✓ Stamens absent — A_0
- ✓ Stamens indefinite in number — A_∞
- ✓ Stamens epipetalous — $\overline{C\ A}$

Gynoecium

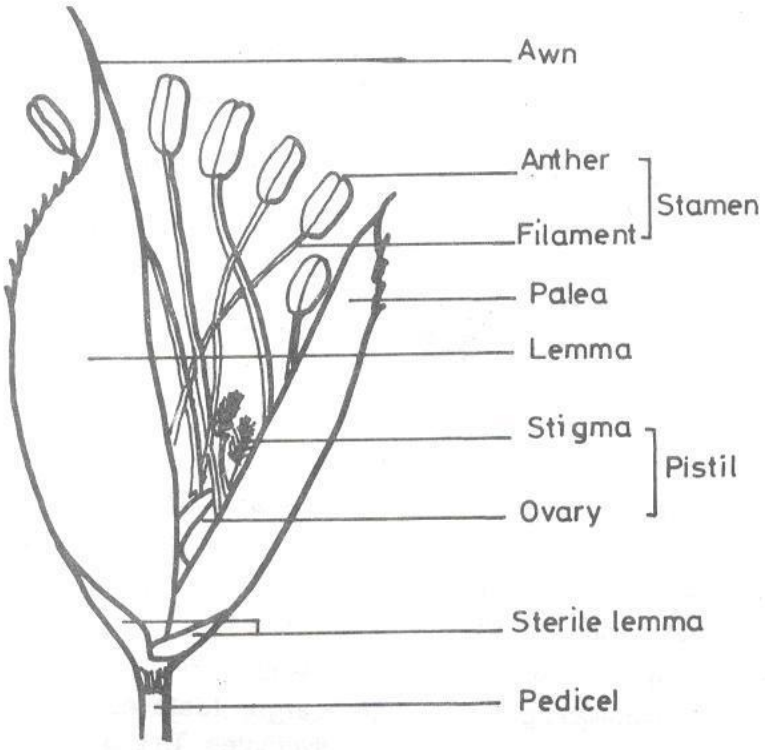
- ✓ Gynoecium — G
- ✓ Two free carpels (apocarpous) — G_2
- ✓ Two fused carpels (syncarpous) — $G_{(2)}$
- ✓ Carpels absent — Go
- ✓ Bicarpellary, syncarpous, superior ovary — $\underline{G}_{(2)}$
- ✓ Bicarpellary, syncarpous, inferior ovary — $\overline{G}_{(2)}$

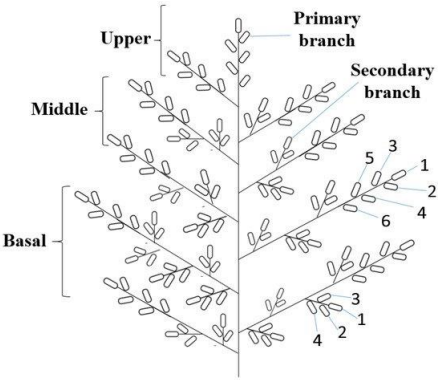
1. Floral structure of Rice (*Oryza sativa*): A self-pollinated crop

https://www.youtube.com/watch?v=-lt_z6FFrd8

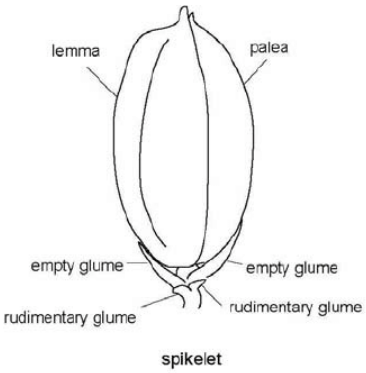
Panicle

- The inflorescence of rice plant, borne on terminal shoot and thus called as panicle.
- It is determinate type and at maturity, it is droopy in nature.

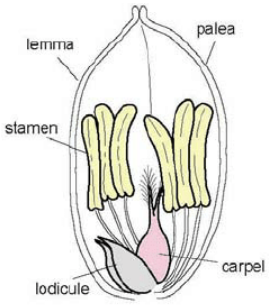




Portion of a rice panicle



spikelet



floret

Spikelet

- A spikelet is the floral unit and consists of two sterile lemmas, a lemma, a palea and the flower.
- Its parts are:
  - Lemma: It is a 5- nerved hardened bract with a filiform extension (of the middle nerve) known as awn.
  - Palea: It is a 3- nerved bract slightly narrower than lemma.
  - Flower: It consists of 6 stamens with two-celled anthers and a pistil with one ovary and two stigmas. The pistil contains one ovule.
  - Fruit: Caryopsis

Floral diagram with floral formula

Exercise:

1. Draw and identify an inflorescence of rice.
2. Draw and identify the different parts of rice flower with floral formula and diagram.

Practical 4. Floral structure of Pigeon Pea (*Cajanus cajan*): A self-pollinated crop

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

https://www.youtube.com/watch?v=-lt_z6FFrd8

Floral morphology and floral structure

Flower : On the basis of flowering two habit groups *viz*.**, determinate and indeterminate are recognized.** In the determinate types**,** the apical buds of the main shoots develop into flowers and flowers occur more or less in the same plane. **In the indeterminate types, flowering duration is longer and flowers occur in axillary racemes spread over considerable length of stem.**

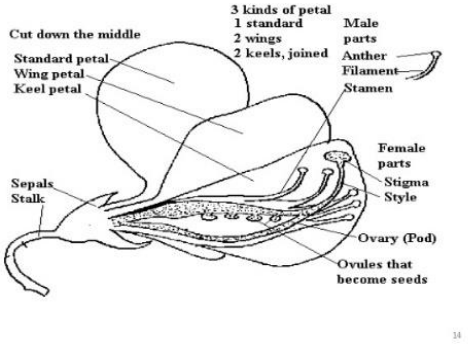
Inflorescence : The flowers are borne in short racemes. Bracts are small with a thick medium nerve enclosing 1-3 young flower buds.

Calyx : Campanulate, with numerous grandular hair and bulbose bases.

Corolla : Zygomorphic, papilionaceous and generally yellow.

Androecium : Stamens are 10, diadelphous (9 + 1).

Gynoecium : The ovary is superior, sub-sessile, and densely pubescent with 2 - 9 ovule. The style is long, filiform and glabrous. The stigma is capitate.



Exercise:

1. Draw and identify an inflorescence of pigeon pea.
2. Draw and identify the different parts of pigeon pea flower with floral formula and diagram.

Practical 5. Floral structure of Maize (*Zea mays*) 2n = 20: A cross-pollinated crop

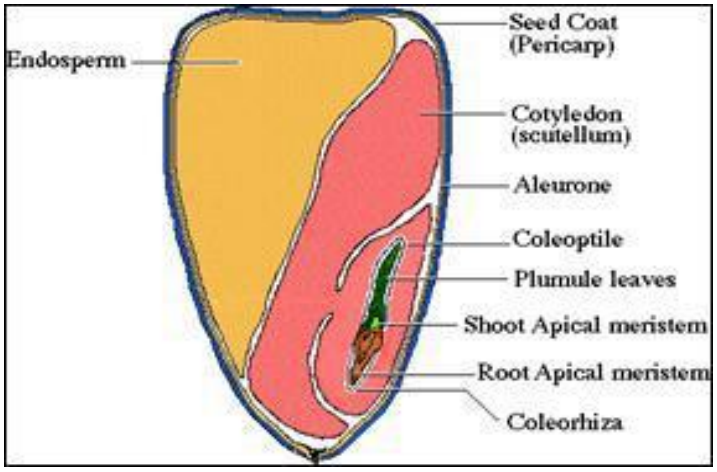
https://www.youtube.com/watch?v=i2brmIfcYvc

Floral Structure

- The corn plant has monoecious flowering structures with pistillate flowers borne on a shoot midway.
- The pistillate inflorescence known as 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 several vertical rows densely crowded and paired sessile female spikelets are present.
- Each spikelet contains two flowers, out of which one is functional giving always even number of rows of karnel on the ear. The lower flower is reduced to lemma and palea which is membranous.
- Rudimentary scale like lodicules may be found showing traces of undeveloped stamens.
- Pistil comprises three carpels, two extending to form silk and third forming the ovule.
- The ovary ends in a thread like structure the silk, which is actually a modified style. The style is long and slender ranging from 35 – 40 cm in length and is receptive all along the length.
- The central axis to tassel is a continuation of the main axis of its stalk.
- Tassel is much branched panicle in which spiklets are arranged on both central axis of the panicle and on the branches in pairs, one sessile and other pedicelled, both being identical in size, shape and structure.
- In each spikelet, there are two florets. Two glumes are common to both the florets. Each floret contains one lemma, one palea, two lodicules and three stamens. Anthers are bloomed and protandrous in nature. Gynoecium is rudimentary.




Tassel Cob Husk Silk



Seed

Exercise:

1. Draw and identify an inflorescence of maize.
2. Draw and identify the different parts of maize flower with floral formula and diagram.

Practical 6. Floral structure of *Cucurbita maxima* L. (2n = 40): A cross-pollinated crop

https://www.youtube.com/watch?v=3aUIN7nYgpg

Floral Structure

Inflorescence: Solitary axillary

1. **Male flower:** ebracteate, pedicellate, incomplete, actinomorphic, unisexual, staminate, pentamerous

   Calyx: sepals 5, gamosepalous, vlvate

   Corolla: petals 5. gamopetalous, valvate, campanulate

   Androecium: stamens 5, arranged in three groups, there are two stamens in two groups and in one group there is only one stamen, Synandrous

   Gynoecium: Absent

2. **Female flower:** ebracteate, pedicellate, incomplete, actinomorphic, unisexual, pistillate, pentamerous

   Calyx: sepals 5, gamocepalous, vlvate

   Corolla: petals 5. Gamopetalous, valvate, campanulate

   Androecium: Absent

   Gynoecium: Tricarpillary, syncarpous, ovary inferior, uniocular, style short, stigma 3

Fruit: pepo

Male flower

Female flower

Exercise:

1. Draw and identify an inflorescence of pumpkin.
2. Draw and identify the different parts of pumpkin flower with floral formula and diagram.

Practical 7: Emasculation and hybridization techniques in self-pollinated crops.

https://www.youtube.com/watch?v=gCfPsuoIftM&t=111s

Selfing and crossing the essential procedures in crop improvement process. The exact procedures used to ensure self and cross-pollination of specific plants will depend upon the floral structure and normal manner of pollination. Generally effecting cross-pollination is a strictly self-pollination species is more difficult than *vice-versa* because for instance preventing self-pollination occurring inside the unopened flowers is cumbersome.

Selfing:

It is important that the breeder, master in selfing and crossing techniques in order to manipulate the pollination according to his/her needs. The exact procedure that he/she may use to ensure self or cross pollination of specific plants will depend upon the particular species with which he/she is working. The structure of flowers in the species determine manner of pollination. For these reasons, the breeder should acquaint himself/herself with the flowering habit of the crop.

In case of wheat, rice, barley, groundnut etc., the plant is permitted to have self-pollination and the seeds are harvested. It is necessary to know the mode of pollination. If the extent of natural cross-pollination is more, then the flowers should be protected by bagging. This will prevent the foreign pollen to reach the stigma. Seed set is frequently reduced in ear heads enclosed in bags because of excessive temperature and humidity inside the bags. In crops like cotton which have larger flowers, the petals may fold down the sexual organ and fasten, thereby pollen and pollen carrying insects may be excluded.

In certain legumes which are almost insect pollinated, the plants may be caged to prevent the insect pollination. In maize, a paper bag is placed over the **tassel** to collect pollen and the **cob (silk)** is bagged to protect from foreign pollen. The pollen collected from the tassel is transferred to the **cob (silk)**.

Emasculation:

Removal of stamens or anthers or killing the pollen of a flower without affecting the female reproductive organ is known as emasculation. In bisexual flower, emasculation is essential to prevent self-pollination. In monoecious plant, male flowers are removed (castor, coconut) or male inflorescence is removed (maize). In species with large flowers e.g. (cotton, pulses), hand emasculation is accurate and adequate.

Methods of emasculation:

1. **Hand emasculation:** It is done before anther dehiscence. It is generally done between 4 to 6 pm one day before anthers dehisce. It is always desirable to remove other young flowers located close to the emasculated flowers to avoid confusion. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed with the help of forceps. Sometimes corolla may be totally removed along with epipetalous stamens *e.g.* sesame.

   In cereals, one third of the empty glumes will be clipped off with scissors to expose anthers. In wheat and oats, the florets are retained after removing the anthers without damaging the spikelets. In all cases, gynoecium should not be injured. An efficient emasculation technique should prevent self-pollination and produce high percentage of seed set on cross-pollination.
2. **Suction Method:** It 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 the suction hose is used to suck the anthers from the flowers. The amount of suction used is very important which should be sufficient to suck the pollen and anthers but not gynoecium. In this method, considerable self-pollination, upto 10% likely to occour. Washing the stigma with a jet of water may help in reducing self-pollination; however, self-pollination cannot be eliminated in this method.
3. **Hot water treatment:** Pollen grains are more sensitive than female reproductive organs in both genetic and environmental factors. In case of hot water emasculation, the temperature of water and duration of treatment vary from crop to crop. It is determined for every species. For sorghum, 42 to 48℃ for 10 minutes is found to be suitable. In case of rice, 10 minutes' treatment with 40 to 44℃ is adequate. Treatment is given before the anthers dehiscence and prior to the opening of the flower. Hot water is carried in thermos flask and whole inflorescence is immersed in hot water.
4. **Alcohol treatment:** It is not commonly used. The method consists of immersing the inflorescence in alcohol of suitable concentration for a brief period followed by rinsing with water. In lucern, the inflorescence immersed in 57% alcohol for 10 second was highly effective. It is better method of emasculation than suction method.
5. **Cold treatment:** Cold treatment like hot water treatment kills the pollen grains without damaging gynoecium. In case of rice, treatment with cold water 0.6℃ kills the pollen grains without affecting the gynoecium. This less effective than hot water treatment.

6. **Genetic Emasculation:** Genetic/cytoplasmic male sterility may be used to eliminate the process of emasculation. This is useful in the commercial production of hybrids in maize, sorghum, pearlmillet, onion, cotton and rice etc., in many species of self-incompatible cases, emasculation is not necessary, because self-fertilization will not take place. Protogyny will also facilitate crossing without emasculation e.g.: pearlmillet.
7. **Use of Gametocide:** Also known as chemical hybridizing agents (CHA), chemicals which selective kill the male gametes without affecting the female gametes. For e.g. sodium methyl arsenate, Zinc methyl arsenate in rice.

Bagging:

Immediately after emasculation the flower or inflorescence is enclosed with suitable bags of appropriate size to prevent random cross pollination.

Pollination:

The pollen grains collected from a desired male parent should be transferred to the emasculate flower. This is normally done in the morning hours during anthesis. The flowers are bagged immediately after artificial crossing.

Tagging:

The flowers are tagged just after bagging. They are attached to the infloresence or to the flower with the help of a thread. The following details may be recorded on the tag with pencil:

1. Date of emasculation
2. Parentage
3. Date of pollination
4. Number of flowers emasculated
5. Signature of emasculator

Note: if flowers are unisexual, the female buds should be protected as emasculated buds are protected by a suitable covering long before stigma receptive.

Precautions taken during emasculation

1. Select appropriate healthy bud
2. Don’t injure other parts of flower
3. Instruments must be well sterilized

4. Use of envelops usually after emasculation just to protect the bud from foreign pollen.
5. Remove too young and too mature buds
6. Stamens should be counted
7. Catch stamens with filament during emasculation
8. 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.
9. Examine by hand lenses and check that stigma is free from pollen
10. Proper tagging is necessary for emasculation.

In Paddy

Emasculation:

It is done in the afternoon on previous day or early in the morning on the day of pollination. The ear just emerged is selected and all spikelet's already opened are clipped the spikelet's which are likely to be opened are selected and six anthers from each spikelet is removed with needle and fine pointed forceps. The emasculated ear after examination with lens covered with perforated butter paper bag and labelled. In mass emasculation method hot water having temperature 42 to 45ºC is carried in thermos flask in the field. The panicle of the proper stage is selected and inserted in the water for 2 to 3 minutes. The flask is unopened spikelet's are clipped off.

Pollination:

It is done on next day morning. Matured anthers are collected from protected male parent in petri dish and dusted on the stigma of emasculated flower with brush and forceps and covered with butter paper bag to protect natural cross pollination.

In pulses

Name of the pulse: Black Gram / *Cicer arietinum* L.) (2n = 16)

Selfing and Crossing

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

i. Buds that will open in one day should be selected for hybridization.
ii. 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.
iii. Take out all the ten anthers by exposing wings and keel. To ensure that all the 10 are removed, no anthers burst.

iv. Observe carefully with magnifying lens for any remains of anthers and bagging is done.

v. 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 emasculated 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 morning between 8am and 11 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.

Exercise:

1. Emasculate the rice plant
2. Self-fertilized the pea

Practical 8: Emasculation and hybridization techniques in cross pollinated crops

https://www.youtube.com/watch?v=ZjWk9AM30Qo

In Maize

Emasculation

The tassels of the female plants are removed immediately as soon as appeared. The process is called as detasseling. It is always done in the morning. Ear shoot which emerging from the leaf sheath is bagged 1 to 2 days below the tip of the previous day of pollination. The tassels of selected male parents is also covered with bag on following day in the morning between 9.00 to 10.00 a.m. pollens from tassel bag is dusted over the silk of the female cob/eat. The bag covered ear shoot is torn and bag from the male parent may be placed over the cob. Care should be taken to avoid contamination of silk with foreign pollens.

Crossing technique Female parent

a. Detassel

b. Cut the tip of the cob before the silks emerge and cover with a butter paper cover.

Male parent

a. Cover the tassel before anthesis begins or as soon as the tassel emerges.

When the silks emerges in the female parent in the form of a brush, pollination is done by transferring the freshly shed pollen cover form the male parent and inserting it over the cob of the female parent after removing the cover from the cob. The details like date of pollination, parentage and breeding programme to be carried out are clearly written by water proof pencil. The date or pollination will be one day later than the date of tasselling. Pollination should be completed within one week of silk emergence. Isolation distance for maize = 400M.

Exercise:

1. Attempt controlled self-pollination and crossing in maize. Make labelled diagrams showing the procedures.

Practical 9: Consequences of inbreeding on genetic structure of resulting populations.

https://www.youtube.com/watch?v=eg7ltl5vll0

Inbreeding is a form of mating system in sexual organism. It implies mating together of individual that are close to each other by ancestral or pedigree relationship.

When the individuals are closely related e.g. Full sib mating, half sib mating. The highest degree of inbreeding is achieved by selfing. The chief effect of inbreeding is to increase homozygosity in the progeny, which is proportionate to the degree of inbreeding. Cross – pollinated and asexually reproducing species are highly heterozygous in nature. These species show a severe reduction in fertility and vigour due to inbreeding (inbreeding depression). It contrast to this hybridization between unrelated strains leads to an increased vigour and fertility (hybrid vigour or heterosis). These two aspects are of great significance in breeding of these species. In fact heterosis and inbreeding depression may be considered as the two opposite sides of the same coin.

Inbreeding Depression: It refers to decrease in fitness and vigour due to inbreeding or it may be defined as the reduction or loss in vigour and fertility as a result of inbreeding.

The most revealing impact of inbreeding is the loss of vigour and the physiological efficiency of an organism characterised by reduction in size and fecundity. For example selfing reduces heterozygosity, by a factor ½ in each generation. In fact the degree of inbreeding in any generation is equal to the degree of homozygosity in that generation. Inbreeding depression results due to fixation of unfavourable recessive genes in F_2, while in heterosis the unfavourable recessive genes of one line (parent) are covered by favourable dominant genes of another parent.

The primary genetic consequence of inbreeding is increased homozygosity (Falconer and MacKay 1996). Two hypotheses for the genetic basis of inbreeding depression are put forth, both of which depend on the idea that homozygosity will increase with inbreeding. Either the overdominance or partial dominance hypotheses are invoked to model the negative consequences of inbreeding (Charlesworth and Charlesworth 1987; Lynch 1991; Karkkainen *et al.* 1999). In the overdominance hypothesis, inbreeding depression is attributable to higher fitness of heterozygotes versus homozygotes for the loci in question (Frankham et al. 2003). For the partial dominance hypothesis, negative fitness consequences for inbred lines are due to the fixation of recessive or partially recessive deleterious alleles (Frankham *et al.* 2003). Current thought favours the latter hypothesis, where inbreeding depression is attributable to many genes of small effect (Keller and Waller 2002, Frankham et al. 2003). However,

distinguishing between the two genetic mechanisms is complicated by linked sets of deleterious recessives that imitate overdominance effects.

Practical 10: Study of male sterility system.

https://www.youtube.com/watch?v=_SIebp6yhZc

1. **Palynology** This is the science involving the study of pollens. The pollen has a very minute structure. It is unicellular and usually round although it may be oval, pyramidal, polyhedral etc. It is provided with two coats-an inner, delicate, cellulose layer called **intine** and an outer tough, cutinised layer called exine or **extine**. The exine is often sculptured or provided with spines, warts etc., occasionally, it is smooth. The exine may have a waxy coating to render the pollen more or less waterproof. Very often, there are some definitely thinner circular spots or slits in the exine called **germ pores** or **slits**. These weak spots are utilized during the germination of the pollen.
2. **Preparation of Acetocarmine Stain ($C_{22}H_2O_{13}$)** It is one of the most widely used stain for pollen study. A mixture of 4 ml glacial acetic acid and 55 ml of distilled water is boiled. A quantity of 1 g of carmine (according to the strength required) is added to 100 ml of the above mixture at about boiling point and then boiled for few minutes. After boiling, the contents are removed from the flame and allowed to cool and filtered in a clean bottle. The filtrate is reddish in colour and known as 1% acetocarmine. Ferric chloride or ferric acetate may be added if necessary, for deep staining and preservation.

Fertility and sterility in A, B, R and TGMS lines

Male sterility is characterized by non-functional pollen grains, while female gametes function normally. It occurs in nature sporadically.

Types of male sterility, maintenance and uses:

Male sterility may be conditioned due to cytoplasmic or genetic factors or due to interaction of both. Environment also induces male sterility. Depending on these factors male sterility can be classified in to

a) Cytoplasmic male sterility (CMS)

b) Genetic male sterility (GMS)

c) Cytoplasmic-genetic male sterility (CGMS)

d) Environmental induced male sterility which is again sub divided in to

i) TGMS (Theromosensitive)

ii) PGMS (Photo sensitive)

A line or ms line: It represents a male sterile line belonging to any one of the above categories. The A line is always used as a female parent in hybrid seed production.

B line or maintainer line: This line is used to maintain the sterility of A line. The B line is isogenic line which is identical for all traits except for fertility status.

R line or restorer line: It is otherwise known as Restorer line which restores fertility in the A line. The crossing between A x R lines results in F1 fertile hybrid seeds which is of commercial value.

Pollen fertility count

a. **Different crop species**

Crop species	Number of pollen grains		Total	Percentage of pollen fertility
	Unstained	Stained		

b. **A, B & R Lines of rice.**

Crop species	Number of pollen grains		Total	Percentage of pollen fertility
	Unstained	Stained		

Practical 11: Methods of calculating mean, range, variance, standard deviation

https://www.youtube.com/watch?v=179ce7ZzFA8

https://www.youtube.com/watch?v=t3p2xhZO29Q

1. Mean: The arithmetic mean is calculated by sum of the total value of the observation divided by number of observation (n).

$$\sum_{i=1}^{n} \frac{y1 + y2 + \cdots Yn}{N}$$

2. Variance: It is a measure of variability and it is defined as the average of squared deviations from the mean or it is also obtained by dividing the sum of squares (SS) by corresponding d.f. (n – 1) to get Mean Sum of Squares (MMS). It is denoted by σ^2.

$$\text{Variance of X} = \frac{\Sigma x2 - \frac{(\Sigma x)2}{N}}{n-1}$$

3. Standard deviation: It is the dispersion of individual values (x) around the population mean

$$\text{SD} = \sqrt{variance} = \sqrt{\sigma 2} = \sigma$$

4. Standard Error (SE): It is the dispersion of family means (x) around the experimental or estimated population mean.

$$\text{S.E} = \sqrt{\text{MSS}} / \text{n} = \text{SD}/ \sqrt{\text{n}}$$

In fact, S.E is the S.D of mean.

It is represented by X ± S. Em

5. Coefficient of variation (CV): When variation has to be compared for different characters, each represented by different units. By converting units of all characters on the same scale, we can compare. CV is expressed as percentage ratio.

$$\text{CV (\%)} = [\text{SD}/ \text{mean}] \times 100$$

Exercise 1:

Calculate the mean, variance, SD, SE and CV of the given data.

Data on seed yield (g/plant) of seven varieties of linseed grown in RBD repeated thrice. The average yield of random samples of 5 plants per plot in all three replicated are presented below.

Average seed yield (g/ plant) of 7 varieties of linseed in 3 replications (r = 3) of RBD.

Variety/ Treatment	R I	R II	R III
A	40	45	40

B	**50**	**50**	**50**
C	**55**	**55**	**50**
D	**60**	**65**	**65**
E	**40**	**40**	**40**
F	**50**	**55**	**55**
G	**65**	**60**	**70**
Total (T_j)	**360**	**370**	**370**

Practical 12: Handling of segregation populations.

https://www.youtube.com/watch?v=Ug7hHtlPmVs

Maintenance of Records

1. Accession Register
2. Germplasm Bank
3. Descriptive blank register
4. Cropping programme
5. Single plant selection register
6. Row test
7. Replicated row test
8. Preliminary / Initial evaluation trial
9. Comparative yield/ yield evaluation trial
10. Multilocation I, II trials.
11. Quality observations Note book
12. Record of crosses
13. F_1 generation
14. F_2 segregation generation note book.

Accession Register

This will contain the details of the seeds/ planting material with regard to receipt date, source, their number, number assigned at the receiving unit, short description of the planting material, to whom sent for evaluation, date, feedback information about the genotype, now how maintained etc., Accession number given by the serial number followed by the year of entry *i.e.* serial 145 in 1991. Then accession number will be 145191 or 91145. It will be mentioned as EC = Exotic collection IC = Indigenous collection.

Proforma for Accession Register

Accession No	Name of variety	Date of Receipt	Source of seed	Source No.	Pedigree record	Description of the material	How disposed to whom sent	Feedback information	Remarks

Standard form of a Field Note Book

Each field note book should contain the following information.

A. Yield Trial

i) First Page

a) Number and title of the project

b) Season of raising the crop

c) Unit under which the trial is being conducted

ii) Second page

a) A full plant of the field showing the location of the trial with the approach path.

b) North East directions should be specified.

iii) Third Page

a. Plan of the experiment

b. Experiment details

- Name of the experiment
- Season
- Number of variants
- Design of the experiment
- Replication
- Size of the plot (Block / Plot / Row, *etc*.)
- Spacing (Between rows and within the row in cm)
- Date of sowing / planting
- Date of harvest
- Name of the Principal Investigator

iv) Fourth page

Details of cultural practices followed for the plot / field

a. Date of ploughing

b. Date of layout of the trial

c. Manurial schedule adopted

- Basal :
- Topdressing :

d. Irrigation schedules with date from life irrigation onwards

e. Plant protection schedules followed

f. Details of intercultural operations A (hoeing, weeding, and earthing up etc.,)

g. Date of harvest

h. Duration of processing till storage

i. Rainfall received during the crop growth

j. General remarks on the seasonal condition prevailed and its effects on crop growth including the occurrence of pests and disease.

v) Fifth page

One page for each variant per replications allotted.

The following information have to be recorded in each page.

- Date of germination
- Date of gap filling
- Initial stand on
- Date of first flowering
- Date of general flowering
- Date of harvest
- Final stand
- Wet weight of grain
- Wet weight of haulms/ straw etc.,
- Dry weight of produce after cleaning
- Yield per ha in kg

The page will also have additional information on observations about the variant, recorded by the breeder in relation to the object of the project.

The fifth page will also contain the following information and their modification depending upon the crop.

e.g. Rice : Date of earhead emergence in the main shoot number of tillers
: Date of earhead emergence in tillers and
: Number of tillers etc.

B. Generation study

This field note book will contain the following information.

a. Plan for the segregation generation

b. Details of the generation

1. Name of the generation study
2. Number of crosses
3. Details of the cross

Cross No. Female parent, Male parent, Number of families, number of seed sown.

4. Length of row
5. Spacing (cm)
6. Date of sowing
7. Dates of harvest
8. Name of the Project Investigator

c. Plan for the segregation generation

d. Details of the generation

1. Name of the generation study
2. Number of crosses
3. Details of the cross

Cross No. Female parent, Male parent, Number of families, number of seed sown.

4. Length of row
5. Spacing (cm)
6. Date of sowing
7. Dates of harvest
8. Name of the Project Investigator

Handling of segregating population in self-pollinated crops

Pedigree Method (Harrington, 1937)

Year	Generation	Activity
I	Crossing block	Crossing between parents
II	F_1	Seeds are space planted and harvested in bulk
III	F_2	2000-5000 plants are space planted from which 200-500superior progenies are selected
IV-V	F_3-F_4	Individual plant to progenies are space planted from which superior progenies are selected
VI-VII	F_5-F_6	Individual plant to progenies are space planted in multirows from which superior plants are selected

VIII	F_7	Preliminary yield trial
IX-XI	F_8-F_{10}	Co-ordinated yield trials, screening for pest and diseases, quality tests are also done.
XII	F_{11}	Seed multiplication for distribution

Bulk Method (Nilsson-Ehle, 1908)

Year	Generation	Activity
I	Crossing Block	Crossing between parents
II	F_1	Raising of F_1. Space planted. At least 20 F_1 plants studied.
III to VI	F_2 to F_6	Planted at commercial seed rate and spacing; generation are harvested in bulk; Allow the environment to act which change the frequency of genotypes; population size 30 to 50000 in each generation.
VII	F_7	Space planted, select 1000 to 5000 plants based on
VIII	F_8	Individual plant to progenies are grown in multirows, 100 to 300 plant to progenies are forwarded.
IX	F_9	PYT conducted; yield form the basis of selection. Quality tests are also conducted.
X-XIII	$F_{10} – F_{12}$	Multilocation yield trial
XIV	F_{13}	Adapted research trial
XV		Best variety released. Simultaneous seed multiplication.

Single Seed Method (Goulden, 1939)

Year	Generation	Activity
I	Crossing Block	Crossing between parents
II	F_1	Space planted and harvested in bulk
III	F_2	F_2 densely planted, from each plant, one random seed selected and bulked
IV to VI	$F_3 – F_5$	Densely planted, from each plant, one random seed selected and bulked
VII	F_6	Space planted. 100 – 500 plants with desirable characteristics harvested separately.
VIII	F_7	Individual plant progenies grown. Desirable homozygous progenies harvested in bulk
IX	F_8	Preliminary yield trial with a suitable check. Quality tests are conducted.
X to XII	$F_9 – F_{11}$	Coordinated yield trial. Disease and quality tests are conducted.
XIII	F_{12}	Seed multiplication for distribution

Exercise

1. Visit the F_2 segregating population which is obtained by selfing of F_1 generation.
2. Collect observations on various economic traits for all the plants in F_2.
3. Observe variability for various characters among F_2 plants.
4. Select best plants having desired traits for yield.
5. Find out mean, SD and CD based on formulae given in earlier chapters.

6. Obtain mean and phenotypic variance in previous F_1 generation. Now, using the following formula, estimate genotypic variance Vg.

$$Vg = V_{F2} - \frac{(Vp1+Vp2+VF1)}{3}$$

   Calculate heritability in broad sense and genetic advance.
7. Observe the genetic gain obtained in F_2 generation from F_1 generation.
8. If you plot a curve for the studied quantitative characters, a normal symmetrical curve depicting all ranges of variability is observed in the population.

Practical 13: Designs used in plant breeding experiments, analysis of Randomized Block Design.

https://www.youtube.com/watch?v=95ZMKW75zvg

The principles of field experimentation entitles to choice of experimental site, size and shape of plots, number of replications, randomization, number of treatments, replication of experiments and field layout.

Randomized Block Design (RBD)

(a) Concept: The RBD is a very simple and flexible field design. It is employed most frequently before, during and after the development of varieties/hybrids for evaluation of their adaptation/performance. The phenotypic variance of test genotypes/ varieties might be partly influenced by soil heterogeneity and/ or by environment. RBD provides a clever and an efficient mechanism for analysis of variance (ANOVA). To account for these source of variation. Three specific points need to be carefully observed.

1. All the treatments i.e. entries/ varieties/ hybrids including checks must have the same set of agronomic and cultural practices.
2. Number of replications should be chosen such that degrees of freedom (d.f.) at error level should not be less than 12.
3. Replications are closely placed in compact blocks for convenience sake. However, there is no restriction to place replication to place replications even far apart, but each replication must be as much compact as possible.

(b) Field Plan: Two factors number of replications and number of treatments are RBD layout. This can be explained with an example. Suppose seven treatments / varieties (entries) of linseed say A to G, the last being a check is to be arranged in 3 replications. Select as far as possible a homogeneous piece of land. Divide it into three compact blocks. Define plot size (variable for crops) and again divide each block into 7 plots. Randomized all the seven entries in each block such that no two contiguous plots have the same variety as shown below.

RBD: layout

C	F	A	D	G	B	E
B	D	E	F	A	G	C
G	E	B	C	F	D	A

(c) Statistical analysis (ANOVA): this can be explained by data on seed yield (g/plant) of seven varieties of linseed grown in RBD repeated thrice as shown earlier in the above layout.

The average yield of random samples of 5 plants per plot in all three replicated and also ANOVA table are presented below.

Average seed yield (g/ plant) of 7 varieties of linseed in 3 replications (r = 3) of RBD.

Variety/ Treatment	R I (x)	R II (x)	R III (x)	Total (T_i)	Mean (g_j)
A	**40**	**45**	**40**	**125**	**41.6**
B	**50**	**50**	**50**	**150**	**50.0**
C	**55**	**55**	**50**	**160**	**53.3**
D	**60**	**65**	**65**	**190**	**63.3**
E	**40**	**40**	**40**	**120**	**40.0**
F	**50**	**55**	**55**	**160**	**53.3**
G	**65**	**60**	**70**	**195**	**65.0**
Total (T_j)	**360**	**370**	**370**	**1100 (GT)**	**52.3 (X) population mean**

ANOVA for seed yield in linseed

Source of variation	Degrees of Freedom (df)	Sum of square (SS)	Mean Sum of Squares (MSS)	Expectation
Replication (r)	**(r-1) = 2**	**RSS**	**RMS**	
Varieties (g)	**(g-1) = 6**	**GSS**	**GMS**	**$\sigma^2 g.\ r + \sigma^2 e$**
Error (r x g)	**(r-1) (g-1) = 12**	**ESS**	**EMS**	**$\sigma^2 e$**
Total	**(rg -1) = 20**	**TSS**		

Various calculations are carried as under:

i. Correction Factor (CF) = **$[\Sigma Ti]^2/N = GT^2/N$**

= $(1100)^2/21 = 57619$

ii. TSS (Total SS) = **$\Sigma X_{ij}^2 - CF$**

= $40^2 + 50^2 + \ldots\ldots..+ 45^2 + 50^2 + \ldots\ldots..+40^2 + 55^2+ 70^2 - CF$

= $59400 - 57619 = 1781$

iii. **RSS (Replication SS)** = **$\Sigma Tj^2/g - CF$**

= $[(360)^2 + (370)^2 + (370)^2/7] - 57619$

= $57628.5 - 57619 = 9.5$

RMS (Replication MSS) = **RSS / (r-1)**

= 9.5/2

= 4.7

iv. GSS (Genotype SS) = **$\Sigma T^2_j / r - CF$**

= $[(125)^2 + (150)^2 + \ldots\ldots\ldots + (160)^2 + (195)^2/3] - CF$

= 59283.3 – 57619

= 1664.3

GMS (Genotype MSS) = **GSS/ (g-1)**

= 1664.3/6

= 277.3

v. ESS (Error SS) = **TSS – (RSS – GSS)**

= 1781 – (9.5 – 1664.3)

= 107.2

EMS (Error MSS) = **ESS / (g-1) (r-1)**

= 107.2 /12

= 8.9

vi. Calculated "F" = **GMS / EMS**

= 277.3 / 8.9

= 31.1

Estimates of variances and heritability

From expectations, we can get the following estimates of various components

$\sigma^2 e$ (Error variance) = EMS = 8.9

$\sigma^2 g$ (Genotypic variance) = (GMS – EMS)/r

= (277.3 - 8.9)/ 3 = 89.4

$\sigma^2 p$ (Phenotypic variance) = $\sigma^2 g + \sigma^2 e$

= 89.4 + 8.9

= 98.3

h^2_{BS} (Heritability in broad sense) = $\sigma^2 g / \sigma^2 p$

= 89.4/98.3 = 0.90 *i.e.,* 90%

Test of significance

Calculated F value greater than table F value at error d.f. (=12) will reflect significance of variance among treatments (genotypes / varieties) at 1% level of significance (P<0.01). A

significant variance will necessitate computation of critical difference / least significant difference (CD/LSD) as follows.

CD 1% for error d.f (=12) = S.Ed x table "t" value (at 1% level)

S.Ed (Standard error of difference) = $\sqrt{}$(2 x (EMS)/r = $\sqrt{}$2 x 8.89/ 3 = 4.219/3 = 1.406

"t" (12 d.f) at 1% = 3.055 (obtained from standard table)

CD = 1.406 x 3.005 = 4.23

Insignificant variance does not require calculation of CD

Interpretation / drawing inferences:

a) Significant GMS (Calculated F> Table F), superscripted with * (at 5% level) or ** (at 1% level) as in the case would indicate significant variation among varieties and vice versa if insignificant.
b) Compare the means using corresponding CD value to locate the highest yielder: in present example variety D is significantly superior over the check (G).
c) Heritability in broad sense (h^2_{BS} = 90%) would mean that the proportion of genetic component σ^2g is 90% indicating that the selection can be safely be exercised. Size of σ^2e shows non-generic or environmental component which is low in this example.

Use of RBD in Plant Breeding: The most widely employed field design. RBD is useful to plant Breeders under following 3 situations.

a) For evaluating new instructions for their suitability (test of adaptation) for early release.
b) For gathering genetic information through biometrical techniques during the course of breeding programme.
c) For local testing (at experimental site) of strains evolved through breeding (tests of adaptation) as also for multilocation testing of varieties in coordinated field trials (tests of adaptability).

Study of genetic variability

Heritability:

$$h^2 = [\sigma^2g/ \sigma^2p] \times 100$$

Genetic advance:

$$GA = [\sigma^2g/ \sigma^2p] \times K$$

K = selection differential which is constant for the known selection intensity (K at 5% selection intensity = 2.06)

PCV (Phenotypic coefficient of variation) =

[Phenotypic standard deviation / Mean] x 100 = [σ_p/ X] x 100

GCV (genotypic coefficient of variation) =

[Genotypic Standard deviation / Mean] x 100 = [σ_g/ X] x 100

Exercise 1.

An experiment was conducted in RBD to assess the yielding abilities of eight paddy varieties. From each replication five panicles were selected at random and the number of filled grains per panicle was observed. The results are tabulated below. Calculate the ANOVA, GCV, PCV, heritability of the given data.

Table: No. of filled grains per panicle of paddy varieties.

Variety	**Replication**		
	I	**II**	**III**
Vaigai	120, 72, 151, 105, 164	98, 102, 139, 117, 107	113, 99, 110, 95, 111
ADT 31	87, 129, 75, 93, 76	100, 94, 102, 131, 136	140, 113, 152, 92, 74
TKM 9	90, 68, 94, 91, 68	122, 154, 122, 83, 97	73, 92, 109, 71, 76
IR 50	69, 68, 96, 85, 88	113, 81, 104, 94, 89	117,79, 86, 75, 88
ACM 5	159, 93, 152, 161, 128	117, 124, 186, 212, 133	126, 127, 132, 116, 132
Karuna	184, 138, 149, 130, 112	148, 126, 128, 152, 215	136, 205, 156, 201, 102
ADT 36	100, 141, 101, 114, 104	83, 140, 118, 103, 137	110, 110, 120, 98, 108
IR 36	102, 104, 100, 96, 85	75, 89, 105, 115, 120	78, 69, 85, 95, 100

Practical 14: Work out the mode of pollination in a given crop and extent of natural out-crossing and Prediction of performance of double cross hybrids.

https://www.youtube.com/watch?v=06nhgUBf1Ag

AIM: To work out the mode of pollination in a given crop and extent of out crossing. 1. To work out the mode of pollination in a given crop.

There are several approaches:

a) Morphological examination of flowers: Mechanism like dioecy, monoecy, protogyny, protandry and cleistogamy are easily detected. They indicate the mode of pollination.

b) Space isolation: Growing single plant of a crop in isolation and recording the seed sit, determines the extent of pollination. Failure o set seeds in isolation proves the crop to be cross pollinated and seed set is indicative of self-pollination.

c) Effects of selfing (inbreeding): Vigour due to inbreeding is common in cross pollinated species while self-pollinated crops show no inbreeding depression.

2) To work out the extent of out crossing: The amount of cross-pollination is determined by planting two strains of the concerned species in a mixed stand. One of these two strains is homozygous for a dominant character, preferably an easily recognizable seeding or other phenotypic character, while other strain is recessive for that character. The two strains are planted in such a manner that each plant of the recessive strain is surrounded by plants of dominant strain to provide abundant pollen. Seeds produced on the recessive strain are harvested and grown in the next generation. The percentage of plant carrying the dominant allele of the character represents the percentage of cross-pollination

Prediction of performance of double cross hybrids.

https://www.youtube.com/watch?v=j9ypV9wW12A

The performance of double cross hybrids can be predicted by comparative evaluation of the predictions based on the performance of single cross.

The method was developed by Jenkins (1934). According to this method, the predicted performance of any double cross is the average performance of the four non-parental single crosses involving the four parental inbred.

For example: If the 4 inbred are I1, I2, I3 and I4. The possible single cross among these inbred would be 6, viz I1 × I2, I2 × I3, I3 × I4, I1 × I3, I1 × I4 & I2 × I4.

These single crosses can combine to produce 3 double crosses, Viz,

(I1 × I2) × (I3 ×I4)

(I1 × I3) × (I2 ×I4)

(I1 × I4) × (I2 × I3)

The performance of any of these double crosses can be predicted from the performance of the four single crosses, not involved in producing that particular double cross.

For example: The performance of double cross (I1 × I2) × (I3 ×I4) would be the average of the performance of the four single crosses (I1 × I3), (I1 × I4), (bv+I2 × I3) and (I2 ×I4)