PRACTICAL MANUAL

ON

PRINCIPLES OF PLANT BREEDING

HFS 105 3(2+1)

B.Sc. (Horticulture) II Semester

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2020

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Syllabus HFS 105 3(2+1): Breeding objectives and techniques in important horticultural crops. Floral biology – its measurement, emasculation, crossing and selfing techniques in major crops. Determination of mode of reproduction in crop plants, handling of breeding material, segregating generations (pedigree, bulk and back cross methods), Field layout, and maintenance of experimental records in self and cross pollinated crops. Demonstration of hybrid variation and production techniques. Hardy Weinberg Law and calculation, male sterility and incompatibility studies in horticultural crops calculation of inbreeding depression, heterosis, heterobeltioses, GCA, SCA, GA, heritability.

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Date: Course Teacher

INDEX

Exercise No. 1

Objective: To study about Plant Breeder Kit's.

Materials required: ……..……

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Items required by the plant breeder and their practical uses.

S. No.	Name of item	Size or type	Used for
1.			
2.			
3.			
4.			
5.			
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7.			
8.			
9.			
10.			
11.			
12.			

Exercise No. 2

Objective: To study about reproduction in plants.

Draw a well-labelled diagram of a mature embryo sac

With a neat, labelled diagram, describe the parts of a typical angiosperm ovule

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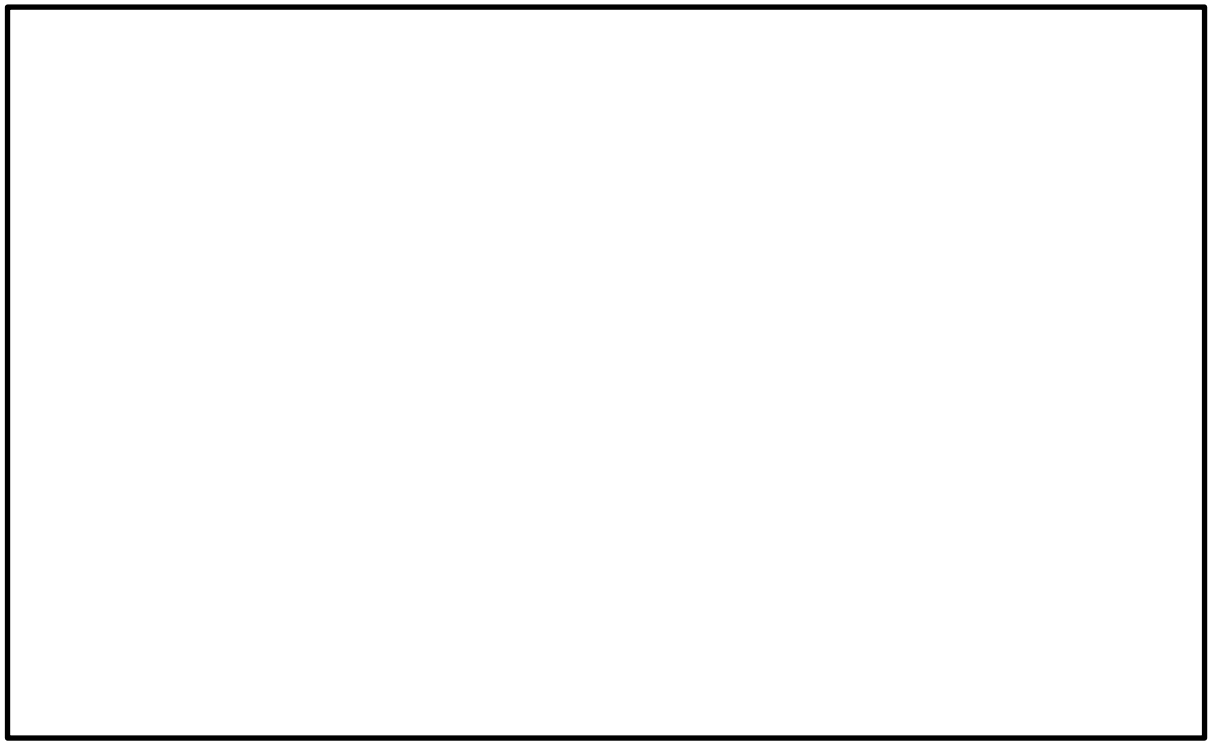
Describe the structure of a pollen grain with the help of a diagram



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Exercise No. 3

Objective: To describe the structure of a typical flower with the help of a well labelled diagram

General description: ……..

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Diagram of a typical flower

Exercise No. 4

Objective: To study classification of Horticultural crop plants based on mode of pollination

S. No.	Common Name	Scientific Name	Family	Chromosome No.
Self-pollinated crop				
Cross-pollinated crop				
Often-cross pollinated crop				

Exercise No. 5

Objective: To work out the mode of pollination in a given crop.

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

Mechanism	Mode of pollination	Crops
Cleistogamy		
Chasmogamy		
Homogamy		
Dichogamy		
Herkogamy		

Space isolation:

Procedure: ……

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Observations:

Name of Crop	Percent Seed Set	Mode of pollination

Inbreeding depression (Effects of selfing):

Procedure: ……

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Observations:

Name of Crop	Inbreeding Depression (%)	Mode of pollination

Extent of out crossing:

Procedure: ……

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Observation: ……

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Result: ……

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Exercise No. 6

Objective: To study the emasculation and pollination technique in tomato

Materials Required: ……

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Scientific name: …….. Chromosome Number – 2n = ……..

Emasculation and Pollination technique Procedure: ……

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Observations:

Date of emasculation	
Time of emasculation	
Date of pollination	
Time of pollination	
Seed set Percentage	

Precautions: ……

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Exercise No. 7

Objective: To study the emasculation and pollination technique in chilli

Materials Required: ……

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Scientific name: …….…. Chromosome Number – 2n = ……..

Emasculation and Pollination technique Procedure: ……

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Observations:

Date of emasculation	
Time of emasculation	
Date of pollination	
Time of pollination	
Seed set Percentage	

Precautions: ……

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Exercise No. 8

Objective: To study the emasculation and pollination technique in Okra

Materials Required: ……

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Scientific name: …….…. Chromosome Number – 2n = ……..

Emasculation and Pollination technique Procedure: ……

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Observations:

Date of emasculation	
Time of emasculation	
Date of pollination	
Time of pollination	
Seed set Percentage	

Precautions: ……

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Exercise No. 9

Objective: To determine viability of pollen grains through staining

Materials required: ……

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Preparation of Acetocarmine Stain ($C_{22}H_2O_{13}$) Procedure: ……

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Fertility of pollen grains Procedure: ……

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Observations:

Slide No.	Total number of pollen grains	Number of pollen grains stained	Number of pollen grains unstained	Pollen fertility (%)

Pollen fertility (%) = Total number of stained pollen/total number of pollen grains) × 100

Results: ……

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Precautions: ……

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Exercise No. 10

Objective: To solve problems based on components of genetic variation

Problem-1: In blackgram, 33 genotypes were evaluated for grain yield in RBD with three replication and following mean square values were obtained for genotypes and error:
MSS treatments = 16.47, MSS error = 2.83, $\bar{X}$ = 11.68. Find out the value of heritability.
Solution: ……

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Problem-2: In a 8 x 8 diallel cross of cotton, following parameters were obtained for fibre length.

D (6.47)	H_1 (3.39)	H_2 (2.86)	F (2.00)	E (0.61)

Calculate heritability in narrow sense:

Solution: ……

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Problem-3: Following estimates were obtained from generation mean analysis:

VF_1 = 0.051, VF_2 = 0.218, D = 0.084. Calculate heritability in broad sense and narrow sense.

Solution: ……

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Problem-4: Calculate broad sense heritability from the following estimates obtained from generation mean analysis. D = 0.842, H = 1.465, E = 0.072

Solution: ……

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Problem-5: The following estimates were obtained from a diallel analysis V_{gca} = 381.26, V_{sca} = 147.43, V_e = 3.65. Calculate heritability in narrow sense.

Solution: ……

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Problem-6: In wheat, following estimates were obtained from Line x Tester analysis.

V_{gca} = 34.15, V_{sca} = 12.27, V_e = 0.32. Calculate heritability in narrow sense.

Solution: ……

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Exercise No. 11

Objective: To solve problems on single crosses

Problem-1: A set of 28 diallel crosses was obtained for evaluation. Find out the number of parents involved in these crosses.

Solution: ……

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Problem-2: A full diallel set of 90 single crosses was made for evaluation. Find out the number of parents involved in these crosses.

Solution: ……

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Problem-3: Find the number of single crosses (with and without reciprocals) that can be constituted if number of parents involved is 14.

Solution: ……

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Exercise no. 12

Objective: To solve numerical based on heterosis and inbreeding depression

Problem-1: In tomato, grain yield of parents P_1 and P_2 and F_1 and F_2 progenies are given below: P_1 = 13.94, P_1 = 22.69, F_1 = 29.38 and F_2 = 15.18. Calculate average heterosis, heterobeltiosis and inbreeding depression.

Solution: ……

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Problem-2: In okta crop, yield data(t/ha) for parents (P_1 and P_2) and F_1 and F_2 progenies for a commercial cultivars and hybrids are given as: P_1 = 10.4, P_2 = 9.2, F_1 = 13.8 and F_2 = 12.1. Commercial cultivar = 13.1. Calculate useful heterosis, standard heterosis and inbreeding depression.

Solution: ……

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Problem-3: A breeder has developed an inter-varietal hybrid of mungbean for early maturity. The hybrid matures in 60 days whereas the parents involved in making hybrid matures in 70 and 65 days and the commercial variety of mungbean matures in 58 days. Calculate the average heterosis, heterobeltiosis and economic heterosis for this case.

Solution: ……

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Exercise No. 13

Objective: To predict the performance of double cross hybrid

Problem-1: The yield performance of the six single crosses developed from 4 inbred lines in maize is given below:

Single crosses	Yield (Kg)
(A x B)	4500
(C x D)	5000
(A x C)	4000
(B x D)	5500
(A x D)	6000
(B x C)	3500

Predict the performance of (A x B) x (C x D); (A x C) x (BxD)

Solution: ……

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Problem-2: In maize six single crosses were made among four inbred lines (A, B, C & D) more evaluated & following yields in tones / ha. Were obtained A x B = 8.8, A x C = 8.9, A x D = 8.4, B x C = 9.2, B x D = 8.0 and C x D = 8.1. Predict the performance of the (A x B) x (C x D); (A x D) x (B x C)

Solution: ……

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Exercise No. 14

Objective: To induce polyploidy through colchicine treatment

Materials required: ……

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Procedure: ……

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Observations:

S. No.	Parameters	
1.	Material treated	
2.	Colchicine concentration used	
3.	Method of application	
4.	Duration of treatment	
5.	Proportion of expected polyploids	
6.	Size of stomata and count	

Result:……

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Exercise No. 15

Objective: To induce mutation through chemical mutagens

Materials required: ……………………………………………………………………………………………

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Procedure: ……………………………………………………………………………………………………

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Observation in laboratory

Crop	
Variety	
Pre-application treatment if any	
Chemical mutagen	
Dose of concentration	
Duration of treatment	

Observation in field

Character	**Treated**	**Control**
Number of seed sown		
Number of seed germinated		
Date when 50 percent germination is over		
Number of lethals after germination		
Chlorophyll mutation		
Dominant mutants		

Precautions: ……

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Exercise No. 16

Objective: To solve problems based on randomized block design

Problem-1: The following are the data recorded for seed yield. Estimate the components of variance.

	Block/Replication			
Entries	**I**	**II**	**III**	**IV**
1	93	97	92	90
2	115	118	123	111
3	120	105	95	126
4	96	119	107	108
5	110	103	115	101
6	122	130	130	111
7	121	124	120	118
8	122	131	129	137

Solution: ……

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Problem-2: An experiment with ten varieties of pea was conducted in RBD with three replications. Estimate the components of variation from the data given below.

Genotypes	Seed weight (g)		
	R_1	R_2	R_3
1	7.8	8.1	7.9
2	11.1	10.9	10.6
3	9.2	9.1	9.2
4	7.9	7.8	7.8
5	8.2	8.2	8.6
6	11.1	11.3	11.5
7	10	10.1	9.8
8	10.2	10.4	10.2
9	9.6	10.1	10.1
10	7.4	7.9	8.3

Solution: ……

Exercise No. 17

Objective: To solve problems based on Hardy-Weinberg law

Problem-1: In a sample of 200 plants of four o' clock plant, 80 were with red flower (RR), 60 with pink flower (Rr) and 60 with white flower. Find out the frequency of genes R and r.

Solution: ……

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Problem-2: In a random mating population of maize, the frequency of a dominant homozygote (RR) is 0.4. find out the frequency of heterozygotes and recessive (rr) individuals.

Solution: ……

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Exercise No. 18

Objective: To collect data on various characters of the plant material under study and maintain a record

Materials required: ……..

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A. Yield Trial

First Page

a. Number and title of the project: ……..

b. Season of raising the crop: ……

c Unit under which the trial is being conducted: ……..

Second page

a. A full plan of the field showing the location of the trial with the approach path (North East directions should be specified)

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Third Page

a) Plan of the experiment: ……..

b) Experiment details

1. Name of the experiment: ……
2. Season:……
3. Number of variants: ……
4. Design of the experiment: ……
5. Replication:……...
6. Size of the plot: ……
7. Spacing:……
8. Date of sowing/planting:……
9. Date of harvest:……
10. Name of the Principal Investigator:……..

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: ……..

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

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g. Date of harvest: ……..

h. Duration of processing till storage: ……..

i. Rainfall received during the crop growth: ……

Complete the accession card for the latest variety of rice/maize.

Variety Accession Card

Department of Plant Breeding, Genetics and Plant Breeding,

RLBCAU, Jhansi

Variety ..... Accession No.……

Genus …… Species ……

Pedigree Record ……. Date of Receipt……..

Specific morphological description ...……..

Source……

Source No.…… Year of discarding ……

Reasons for discarding…….

Remarks……

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2) Record data in a field notebook for a given crop.

Data on various plant characters of …….

S. No.	Strain	Seedling count	Seedling vigour	Days to 50% flower	Days to 50% maturity	Plant height	Number of primary branches	Number of secondary branches	Pod length	Number of seeds per pod	Seed weight	Yield

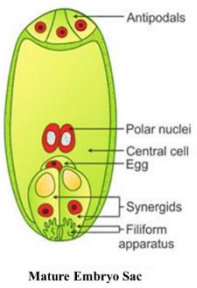
PLANT BREEDER'S KIT

Name of item	Size or type	Used for
Scissors	Small or medium size	Clipping of unwanted parts such as buds, awn etc.
Forceps	Long pointed	Emasculation and transfer of pollen to the stigma.
Needles	Long with plastic handle	Opening small flower buds for emasculation.
Magnifying glass	Small or medium size	Ensuring absence of pollen in emasculated bud.
Brushes	Small and medium	Placing pollen on the stigma during crossing.
Bags	Parchment paper bag, tissue paper bag, brown paper bags, muslin cloth bags	Covering inflorescence or bud before and after pollination.
Tags	Paper tags Tin foil	Labelling plants.
Weighing balance	Small size	Weighing large number of samples with small quantity.
Measuring tape	100 meters	Measuring dimensions of field.
Meter scale Pencil	1 meter ordinary	Measuring plant height recording observations, writing labels
Field note book	Standard	Recording field and laboratory observations.
Spirit or alcohol	Methylated	Sterilizing scissors, needles, forceps', brushes and hands

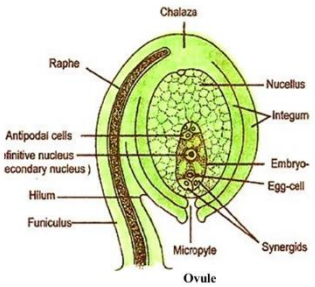
REPRODUCTION IN PLANTS

Sporogenesis: Process of production of microspores and megaspores is known as sporogenesis. Microspores are produced in anthers i.e. microsporogensis, while megaspores are produced in ovules i.e. megasporogenesis

Microsporogenesis: The sporophytic cells in the pollen sacs of anther which undergo meiotic division to form haploid i.e., microspores are called microspore mother cell (MMC) or pollen mother cell (PMC) and the process is called microsporogenesis. Each PMC produces four microspores and the microspores mature into pollen grains mainly by thickening of their walls.



Mature Embryo Sac



Ovule

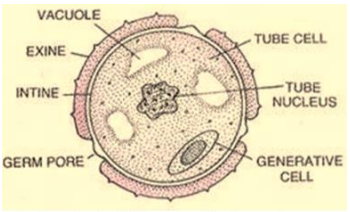
Megasporogenesis: A single sporophytic cell inside the ovule, which undergo meiotic division to form haploid megaspore, is called megaspore mother cell (MMC) and the process is called megasporogenesis. The resultant of the meiosis of the megaspore mother cell is four haploid megaspores. Of the four cells that form, only one functional megaspore is functional while the other degenerate.

Gametogenesis: The production of male and female gametes in the microspores and megaspores, respectively, is known as gametogenesis.

Microgametogenesis: Microgametogenesis refers to the production of male gametes or sperm. On maturation of the pollen, the microspore nucleus divides mitotically to produce a small generative and a large vegetative or tube nucleus. The pollen is generally released in this binucleate stage. The pollen when reaches over the stigma, this step is known as pollination. After the pollination, the pollen germinates. The pollen tube enters the stigma and travels down the style. The generative nucleus at this phase undergoes another mitotic division to produce two male gametes or sperm nuclei. The pollen along with the pollen tube possessing a pair of sperm nuclei is called microgametophyte. The pollen tube enters the embryo sac through micropyle and discharges the two sperm nuclei.

Megagametogenesis: The nucleus of the functional megaspore divides mitotically to form two nuclei which move to the opposite poles, forming the 2-nucleate embryo sac. Two more sequential mitotic nuclear divisions result in the formation of the 4-nucleate and later the 8-nucleate stages of the embryo sac. After 8-nucleate stage, cell walls are laid down leading to the organization of the typical female gametophyte or embryo sac. Six of the eight nuclei are surrounded by cell walls and organized into cells; the remaining two nuclei, called polar nuclei are situated below the egg apparatus in the large central cell.

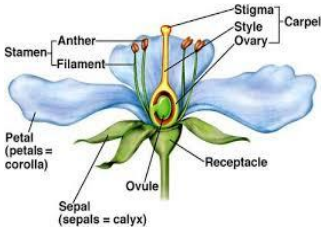
There is a characteristic distribution of the cells within the embryo sac. Three cells are grouped together at the micropylar end and constitute the egg apparatus. The egg apparatus, in turn, consists of two synergids and one egg cell. The synergids have special cellular thickenings at the micropylar tip called filiform apparatus, which play



Section of a 2 celled pollen grain of an angiosperm

an important role in guiding the pollen tubes into the synergid. Three cells are at the chalazal end and are called the antipodals. The large central cell, as mentioned earlier, has two polar nuclei. Thus, a typical angiosperm embryo sac, at maturity, though 8-nucleate is 7-celled. (The exact number of nuclei and their arrangement varies from one species to another.)

FLORAL STRUCTURE



Typical flower structure: Flowers are, the sites of sexual reproduction in flowering plants. A flower usually consists of sepals, petals, stamens and pistil. A flower containing both stamens and pistil is a perfect or hermaphrodite flower. If it contains stamens but not pistil, it is known as staminate while a pistillate flower contains pistil but not stamens, sepals, petals each collectively called calyx and corolla, respectively; both collectively called perianth. Stamens are collectively called androecium and pistils or carpels are collectively called gynoecium. Stamens consists of stalk, connective and anther while carpels consist of stigma, style and ovary. Staminate and pistillate flowers occur on the same plant in a monoecious species such as maize, castor, coconut etc. but in dioecious species, staminate and pistillate flowers occur on different plants e.g., papaya, date palm, hemp etc. the male gamete is produced in stamen, while the pistil produces the female gametes.

CLASSIFICATION OF CROPS BASED ON MODE OF POLLINATION

Pollination refers to the transfer of pollen grain from anthers to stigmas. Pollen from an anther may fall on the stigma of the same flower leading to self-pollination or auto gamy. Sometimes pollen from an anther may fall on the stigma of another flower of different plants leading to cross pollination or allogamy. Sometimes pollen from an anther fall on the stigma of the anther flower of same plant leading to the geitonogamy.

Self-Pollination: It is transfer of pollens from and to the stigma within the same flower, is always found in bisexual flower. In most of these species self-pollination is not complete and cross- pollination may occur up to 5%. There are various mechanism/contrivances that promote/facilitate self-pollination.

Bisexuality: Male and female sexual organs present in the same flower e.g., wheat, rice, groundnut, etc.

Homogamy: Male and female sexual organs mature at the same time e.g., wheat, groundnut, etc.

Cleistogamy: In this condition flowers do not open at all and ensure complete self-pollination e.g., oat, barley, wheat, grasses, etc.

Chasmogamy: In some species, flower open but only after pollination has taken place. e.g. Barley, wheat, oat, and many cereals.

- In crop like Tomato and Brinjal stigma are closely surrounded by anthers, hence pollination occurs after opening of flower but the position of anther in relation to stigma ensure self-pollination.
- In crop like pea, bean, soybean, the flower open but stigma and anther are hidden by floral organ and ensures self-pollination.
- In few species, stigmas become receptive and elongate through staminal column, ensures self-pollination.

GENETIC CONSEQUENCES OF SELF-POLLINATION:

- It leads to a very rapid increase in homozygosity; therefore, self-pollinated species are highly homozygous in nature.
- Self-pollinated species do not show inbreeding depression, exhibit considerable heterosis.

Cross-Pollination: The transfer of pollen from a flower to the stigma of the other flower of different flower plant. In cross pollinated species pollination may be brought about by wind, water insect or animals. Wind (anemophily), water (hydrophily), insect (entomophily) and animal (zoophily). In most of the cross-pollinated sp. viz., bajra, maize, sunflower, alfalfa, castor, cross pollination is not complete, and self-pollination may occur 5-10%. There are several mechanism contrivances that facilitate cross pollination.

Dicliny (Unisexuality): It is a condition in which flower is either staminate or pistillate.

a) Monoecy: Staminate and pistillate flowers occur in the same plant either in the same inflorescence. e.g. Mango, banana, coconut or in the separate inflorescence. e.g. Maize, Cucurbit, Strawberry, etc.
b) Dioecy: The male and female flowers are present on different plants i.e. the in such species are male or female i.e. sec is governed by a single gene. e.g. Papaya, hemp, date, palm, etc.

Dichogamy: Anther and stigma of hermaphrodite flower mature at different time, facilitating cross pollination.

a) Protogyny: Gynoecium matures earlier than the androecium e.g. bajra.
b) Protandry: Androecium matures earlier than gynoecium. e.g. marigold, maize, cotton, etc.

Heterostyly: Different length of style and filaments e.g. linseed.

Herkogamy: Presence of physical barrier or mechanical obstacles between the anther and stigma ensures cross pollination. e.g. Milkweed (Madar) *Calotropic gigantia*.

In lucerne or alfalfa stigma are covered by waxy film and it does not become receptive unless this waxy film is broken by honeybees.
A combination of two or more of the above mechanism may occurs in some species, e.g., maize, - Monoecy and Protandry.

Self –Incompatibility: It refers to the failure of pollen from a flower to fertilize the same flower or other flowers on the same plant. It may be saprophytic or gametophytic e.g., mustard, tobacco, sunflowers, reddish.

Male Sterility: It refers to the absence of functional pollen grains in hermaphrodite flower.

Genetic Consequences of Cross Pollination:

1) It preserves and promotes heterozygosity in population.
2) Cross pollinated species shows inbreeding depression and considerable heterosis.
3) Usually hybrid and synthetic without reducing heterozygosity.

Often-cross Pollination: In this type plants are self-pollinated; however, the extent of cross pollination often exceeds 5 to 50 % such species are generally known as often cross-pollinated species. e.g. jowar, cotton, safflower, *arhar*, etc. The genetic architecture of such crop is intermediate between self- and cross-pollinated crops and breeding methods suitable for both of them may be profitably applied.

MODE OF POLLINATION IN CROP AND EXTENT OF NATURAL OUT-CROSSING

There are some natural mechanism favoring a particular type of pollination which are of great use to differentiate pollinating system in crop plants. However, some other methods are also available which can be appropriately applied with natural devices for this purpose. There are several approaches to work out the mod e of pollination in a given crop:

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

Cleistogmous condition:	Self-pollinated
Chasmogamous condition:	Self-pollinated
Homogamy:	Self-pollinated
Dichogamy:	Cross-pollinated
Herkogamy:	Cross-pollinated

Space isolation: If a single plant is raised in isolation normal seed setting will indicate self-pollination while erratic or no seed set means flowers need pollen from other sources. Thus, cross pollination can easily be identified by isolation planting.

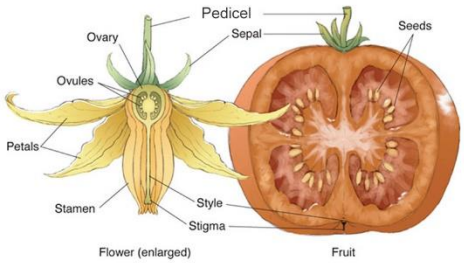
Inbreeding depression (Effects of selfing): If selfing of plants leads to considerable loss of vigor, cross pollination may be anticipated. Conversely, little or no loss of vigor indicate self-pollination in plants.

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 recessive strain may be planted preferably in a polycross nursery in isolation along with dominant strain. Collect the seeds produced on the recessive strain and grow them in the next generation. The percentage of plant carrying the dominant allele of the character represents the percentage of cross-pollination.

HYBRIDIZATION TECHNIQUE IN TOMATO

Emasculation is usually done one day prior to anthesis/flower opening. At this stage, the sepals have started to separate, and the 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 forceps between the sepals to grip the base of the anthers and / or petals which are then removed by a firm but steady pull. If anthers seem reluctant to part company from flower receptacle as a group, it is advisable to remove a single one first by



careful manipulation of the forceps. Following this, the remaining four may be gripped firmly without any fear of damaging the style. Pollen is best applied in experimental crosses by slitting the inside of the anthers of mature flowers of the male parent with the forceps in such a way that a small amount of pollen is collected at the tip of the forceps. This can 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. Pollen may be collected in large amounts by inverting the mature flower and tapping pollen into the thumbnail (Watts, 1980). Protection of pollinated flowers by wrapping with cotton or small pollination bags is essential.

HYBRIDIZATION TECHNIQUE IN CHILLI

Flowers are emasculated in bud stage. Pollen is transferred to the stigma either from mature undehisced anthers by scooping it out through the lateral sutures with the needle or by touching a freshly dehisced anther to the stigma with the forceps. Hands and tools (a pair of sharp-pointed forceps, a needle, a pair of scissors) are washed with 95% ethyl alcohol. A roll of cheese cloth, some light weight cotton string and balls of different colours of thread are also needed. Pollinated flowers are identified by loosely typing coloured thread around the delicate pedicels, preferably enclosing a leaf petiole for protection. Different colours of string can be used for different crosses on the same plant, and white for the selfs. Pollinated flowers are protected from bees by a double layer of cheese cloth, loosely wrapped around the branch, enclosing leaves and flowers, and securely fastened. Appropriately marked plastic labels describing the cross, the date, are attached to a bamboo stake marking the chosen plant. Pollinated flowers are periodically checked and the cheese cloth are removed in 4-6 days. Fruits normally mature in about 45 days.

HYBRIDIZATION TECHNIQUE IN OKRA

Emasculation: The buds that will open next day, are selected in female parent and emasculation is carried out by removing the androecium along with the corolla. These emasculated buds are covered with butter paper pockets to avoid cross pollination and also for easy identification of emasculated flower for pollination. The emasculation should be carried out daily from 2-00 to 6-00 pm. Care must also be taken to remove the un-emasculated flowers as per treatment and during emasculation to avoid genetic contamination in the crossed ones.

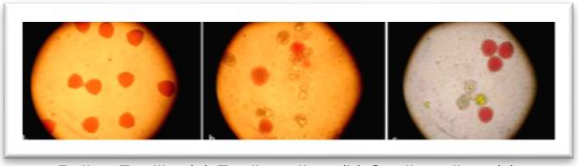
Pollination: The just opened flowers are picked from the male parent in a separate brown paper pockets and used for crossing of emasculated flowers. One male flower can be used to pollinate four to eight female emasculated buds as par the pollen load and after crossing different colour thread are tied to the pedicel of the crossed buds for easy identification of the crossed bud. Pollination should be carried out between 8-00 am to 4-00 pm.

POLLEN VIABILITY TEST

The branch of science, which deal with the study of pollen grains is called palynology. Pollen grains or microspores develop from microspore mother cells through the process called microsporogenesis, which involves meiosis.

The study of pollen grains is important from several point of views such as pollen viability, fertility, physiology, development and incompatibility. Pollen viability refers to the ability of a pollen grain to germinate and produce male gametes. Pollen grains of different crops are viable for a short period after release from anthers. Major causes for the loss of pollen viability are:

1. Pollen may desiccate in air
2. Deficiency of respiratory substances
3. Loss of membrane permeability
4. Inactivation of enzymes and growth hormones
5. Chromosomal aberrations



Pollen Fertility (a) Fertile pollen; (b) Sterile pollen; (c) Partially fertile pollen

Several chemical stains may be used for testing pollen viability such as acetocarmine, acetoorcein, potassium iodide, triphenyl tetrazolium chloride, Evan's blue etc.

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 2 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 2% acetocarmine. Ferric chloride or ferric acetate may be added if necessary, for deep staining and preservation.

Pollen viability test:

1. To determine the pollen fertility fully developed floral buds will be collected randomly from each plant and the anthers will be squashed in 2% aceto-carmine stain on a micro slide and examine under a light microscope using 100x magnification.
2. The viable pollen grains will be deeply stained, whereas dead pollen will be unstained and will be shriveled and irregular.
3. Five microscopic fields for each sample will be examined. Mean of the five microscopic fields will be calculated and the proportion of fertile pollens will be expressed in percentage.
4. Percent pollen fertility will be calculated as

   Pollen fertility (%) = $\frac{\text{Total number of stained pollen}}{\text{Total number of pollen}} \; x \; 100$

   [Normal deeply stained pollen grains were counted as viable, while weakly stained were recorded as non-viable].

COMPONENTS OF GENETIC VARIATION

Genetic variation: It is inherent variability which remains unaltered by environmental conditions. This type of variability is more useful to a plant breeder for exploitation in selection or hybridization R.A. Fisher (1918) was the first to divided the genetic variation in to three types: Additive, Dominance and Epistatic. Write (1935) suggested the partitioning of genetic variation in to two components viz., additive and non-additive (dominance and epistatic components) of which only additive components contributes to genetic advance under selection. Mather (1949) divided the phenotypic variance in to three components namely:

Heritable fixable (additive variance),
Heritable non-fixable (dominance and epistatic components and),
Non-heritable non-fixable (environmental variation).

The above discussion may be summarized as follows:

VP = VG + VE
VG = VA + VD + VI
VI = VAA + VAD + VDD, Where

VP = Phenotypic variance
VG = Genotypic variance
AV = Additive variance
VD = Dominance variance
VI = Epistatic variance
VAA = Additive x Additive variance
VAD = Additive x Dominance variance
VDD = Dominance x Dominance variance

Genetic advance: Improvement in the mean genotypic value of selected plants over the parental population is defined as genetic advance and is the measure of genetic gain under selection. The success of genetic advance depends on the three main factors as described below:

Genetic variability: The magnitude of genetic variability present in the base population has important bearing on the genetic advance and vice-versa.

Heritability: The genetic advance is high with characters having high heritability and vice-versa.

Selection intensity: The proportion of plants or families selected is called selection intensity. High selection intensity generally gives better results than low selection intensity.

Computation of genetic advance (GA): Its computation is based upon knowledge of genetic gain and selection differential.

Genetic gain: The difference between mean phenotypic value of the progeny of selected plants and the base population is known as genetic gain and is denoted by R.

R = XP – XO Where,
XP = mean phenotypic values of the progeny of selected plants,
XO = base population.

Selection differential: The difference between mean phenotypic values of selected plants and that of parental population is termed as selection differential and is denoted by K.

K = XS – XO Where,
XS = mean phenotypic value of selected plants,
XO = mean phenotypic value of parental population. Hence,

G.A. = K x H x SD (P)
= K x Vg / Vp x $(Vp)^{1/2}$ Where,
K = selection differential,
H = heritability,

SD (P) = phenotypic standard deviation of base population.

Interpretation of results:

i. If the value of genetic advance is high, it shows that the character is governed by additive genes and selection will be rewarding for the improvement of such trait.
ii. If the value of genetic advance is low, it indicates that the character is governed by non-additive and heterosis breeding may be useful.

Heritability: Heritability is an index of the transmission of characters from parents to their offspring. Heritability is of two types, viz., broad sense heritability and narrow sense heritability.

Broad sense heritability: The broad sense heritability, from different materials, is estimated in different ways. From replicated data of several genotypes, heritability is calculated as follows:

Heritability (bs) = $\frac{VG}{VP}$ x 100

Where,

VG = genotypic variance, and
VP = phenotypic variance

From generation mean analysis, the heritability in worked out with the help of following formula.

Heritability (bs) = $\frac{VF2-VF1}{VF2}$ x 100

Where,

VF_1 = Variance of F_1 progeny
VF_2 = Variance of F_2 progeny

Narrow sense heritability: It is calculated in different ways from different breeding materials and biometrical techniques.

a. From diallel analysis: The following formula is used for calculation of heritability (ns):

Heritability (ns) = $\frac{\frac{1}{2}D+\frac{1}{2}H1-\frac{1}{2}H2-\frac{1}{2}F}{\frac{1}{2}D+\frac{1}{2}H1-\frac{1}{4}H2-\frac{1}{2}F+E}$ x 100

Where,

D = Variance due to additive effect of genes
H_1 = Variance due to dominance effect of genes
$H_2 = H_1 [1- (u-v)^2]$, where u and v are proportion of positive and negative genes in the parents.
F = The mean of Fr over the array, where Fr is the covariance of additive and dominant effects in a single array.
E = Expected environmental component of variance

Verhalen and Murray (1969) proposed the following formula for calculation of heritability from F_2 generation of a diallel cross.

Heritability (ns) = $\frac{\frac{1}{4}D}{\frac{1}{4}D+\frac{1}{16}H1-\frac{1}{8}F+E}$ x 100

b. From partial diallel analysis: The heritability is calculated by the following formula:

Heritability (ns) = $\frac{2Vgca}{2Vgca+V\ sca+VE}$ x 100

Where,

Vgca = Variance due to general combining ability
Vsca = Variance due to specific combining ability
VE = Error variance

c. From Line x Tester analysis: The heritability is calculated by the following formula:

Heritability = $\frac{Vgca}{Vgca+Vsca+VE}$ x 100

d. From generation means: Mather (1949) and Warner (1952) have suggested separate methos of calculating heritability from generation means as given below:

Heritability (ns) as per Mather (1949) = $\frac{D}{D+H+E}$ x 100

Where,

D = Additive variance
H = Dominance variance
E = Error variance

Heritability (ns) as per Warner (1952) = $\frac{\frac{1}{2}D}{VF2}$ x 100

Where,

VF_2 = Variance of F_2 generation.

SINGLE CROSSES

In plant breeding and genetics, single crosses are made for various purposes such as to develop hybrid cultivar, estimate combining ability, heterosis and inbreeding depression. Single cross refers to a series of crosses each involving two parents.
All possible single crosses = n (n-1)/2
Where,

n = number of parents involved

ESTIMATION OF HETEROSIS AND INBREEDING DEPRESSION

Heterosis refers to the superiority of F_1 hybrids over its parents. The term hybrid vigour is used as synonym for heterosis.

Estimation of heterosis: Heterosis is estimated in three different ways, vis.

Average heterosis: Heterosis is measure over the mean value of two parents involved in the crops. Heterosis is estimated as follows:
Average heterosis = $[(F_1 – MP) / MP] \times 100$
Where,

F_1 = Mean value of F_1
MP = Mean value of two parents.

Heterobeltiosis: When the heterosis is estimated over the superior or better parent it is referred to as heterobeltiosis. It is workout as follows:

Heterobeltiosis = $[(F_1 - BP) / BP] \times 100$, Where,

F_1 = Mean value of F_1
BP = Mean value of better parents.

Useful heterosis: The term useful heterosis was used by Meredith and Bridge (1972). It refers to the superiority of F_1 hybrid over the standard commercial check variety. It is also called economic heterosis. It is estimated as follows:

Useful heterosis or economic heterosis = $[F_1 – CC] \times 100$
Where,

F_1 = Mean value of F_1
CC = Commercial cultivar.

Inbreeding depression: Inbreeding depression may be defined as the reduction or loss in vigour and fertility as a result of inbreeding. Inbreeding depression is estimated by using the following formula:

Inbreeding depression (ID) = $[F_1 – F_2] / F_1 \times 100$

PREDICTION OF PERFORMANCE OF DOUBLE CROSS HYBRIDS

Double cross hybrid: Single crosses are used to predict the performance of double cross hybrid. The yield of a double cross can accurately be predicted from the mean yield of the four non-parental single crosses. The average performance of single crosses A x C, A x D, B x C and B x D is used to predict the performance of the double cross (A x B) and (C x D). the use of single cross performance for the prediction of double cross performance has become a standard breeding procedure. Multi-locational or multi seasonal testing is desirable to predict the performance of a double cross hybrid. The predicted yield is worked out as follows:
Predict yield of (A x B) x (C x D) = [(A x C) + (A x D) + (B x C) + (B x D)] ¼.

INDUCTION OF POLYPLOIDY

Colchicine has the property of arresting and breaking the spindle, so that a cell division without cell wall formation may be affected leading to doubling of chromosome number. The concentration of aqueous solution of colchicine may vary from 0.01% to 0.50% and the treatment may be given in one of the following manners,

- Seed treatment may be mainly given by soaking seeds for different duration in aqueous solution of colchicine.
- Injection of colchicine solution may also be given at seedling stage so as to inject solution into cortex tissue with the help of a hypodermic needle.
- Axillary bud treatment in also effective. Since bud is meristematically active, placing cotton soaked in colchicine on the bud and continuous dropping of solution on cotton leads to induction of polyploidy in the branch arising from the treated bud.
- Shoot apex treatment is brought about just like bud treatment and is fairly effective but the shoot apex should come in

direct contact of the solution. In order to facilitate this young leaves covering the shoot apex may be removed.

Colchicine is an alkaloid extracted from seed and corm of *Colchicum autumnale*. This action of colchicine and its use inducing polyploidy was first studied in 1930's. the successful doubling of chromosome number was described for the first time by Levan 1938.

Species-A X Species-B
(AA) (BB)
Diploid ↓ Diploid
AB
Diploid F_1 hybrid sterile
↓ Colchicine
AABB
Amphidiploid tetraploid (fertile)

Procedure: Colchicine can be applied in the following ways:

1. **Capping Method:** An aqueous solution of colchicine is sucked into a bent glass tube fitted with a rubber teat. The cut end of the tiller is then inserted into the solution contained in the tube and held in a position by a piece of wire. The colchicine solution is retained throughout the time of treatment.
2. **Wilk method:** Five to six days old seedling are placed on filter paper wilks that are dipped in 0.5 ml colchicine solution of varying strength (0.05-1%) in test tubes, for 24 hr. or more. Thus, cotyledons and shoot apices have no direct contact with the solution. Then after rinsing them in distilled water the treated seedlings are transferred to fresh wilks in tubes containing distilled water for a week before transplanting them in soil.
3. **Cotton wool method:** The young plants are wrapped up at the base with cotton soaked in colchicine solution and plants are covered with an inverted glass jar to prevent evaporation of the solution.
4. **Lanolin-colchicine paste method:** An aqueous solution of colchicine of specified concentration in lanolin paste, dipped on to a cotton pad is topically applied to the growing points or meristematic tissues (root or shoot tip) of a seedling for a specified time. Colchicine solution is either applied after regular intervals or is supplied continuously through a hanging thread from the tube fixed conveniently over the twig to prevent drying.

INDUCTION OF MUTATION

Physical Mutagens: Physical mutagens include various types of radiation, viz., X-rays, gamma rays, alpha particles, beta particles, fast and thermal (slow) neutrons and ultra-violet rays.

Radiation	Main properties	Mode of action or changes caused
X – rays	S.I., penetrating and non-particulate	Induce mutations by forming free radicals and ions. Cause addition, deletion, transitions and transversions.
Gamma rays	S.I., very penetrating and Non-particulate	Induce mutations by ejecting atoms from the tissues. Cause all types of changes as above.
Alpha Particles	D.I., particulate, less penetrating and positively charged.	Act by ionization and excitation. Cause chromosomal and gene mutations.
Beta Rays Particles	S.I., particulate, more penetrating than alpha particles and negatively charged.	Act by ionization and excitation. Cause chromosomal and gene mutations.
Fast and Thermal Neutrons	D.I., particulate, neutral particles, highly penetrating.	Cause chromosomal breakage and gene mutations.
Ultraviolet Rays	Non-ionizing, low penetrating	Cause chromosomal breakage and gene mutations.

Chemical Mutagens: The chemical mutagens can be divided into four groups, viz. 1) alkylating agents, 2) base analogues, 3) acridine dyes, and 4) others.

Group of mutagens	Name of chemical	Mode of action
Alkylating Agents	Ethyl methane Sulphonate	AT to GC Transitions
	Methyl methane Sulphonate	Transitions
	Ethyl Ethane Sulphonate	GC to AT Transitions
	Ethylene Imines	Transitions
Base Analogues	5 Bromo Uracil	AT to GC Transitions
	2 Amino Purine	AT to GC Transitions
Acridine Dyes	Acriflavin, Proflavin	Deletion, addition and frame shifts
Others	Nitrous Acid	AT to GC Transitions

	Hydroxylamine	GC to AT Transitions
	Sodium Azide	Transitions

INDUCE MUTATION IN CROP PLANTS

Materials required: Seed, pollen grain, buds, distilled water, X-ray, gamma ray, EMS, petri plates, conical flask, beaker, pipette, glass rods, measuring cylinder, stop watch, etc.

Procedure:

1. Treat the seed or dormant propagating material with 10kR, 20kR, 40kR, 60kR, 80kR and 100kR of X-ray or gamma ray. Alternatively, treat the seed or propagating material with EMS at varying concentrations for 1, 2 and 4 hours.
2. Seeds may also be presoaked in the distilled water for different hours depending upon the seeds, to initiate biochemical reactions.
3. Intermittent shaking should be given to ensure uniform exposure of the chemicals. The chemical should be drained after the treatment time is over.
4. The seeds should be washed thoroughly in running tap water, immediately for not less than 30 minutes. After washing, the seeds should be dried in between the filter paper folds.
5. Do dense planting in the field along with a control plot.
6. Record observations on germination percentage, chlorophyll mutants and dominant mutants
7. Periodical observation on germination up to 10-15 days is needed. From the germination percentage, we can assess the LD50 dose.

EXPERIMENTAL DESIGNS USED IN PLANT BREEDING

Experimental designs and its principle: Designs are various types of plot arrangements, which are used to test a set of treatments to draw valid conclusions about a particular problem. There are three basic principles of experimental designs viz; replication, randomization and local control. All these principles help in reducing the experimental error and thus make the experimental more efficient.

Types/choice of experimental designs: The choice of designs depends on three main factors viz;

- Number and nature of treatments under study,
- Objectives of the experiment, and
- Available resources. Different types of experiment, designs are used under different situation as given below:
- When experimental material is limited and homogeneous. Completely randomized designed (CRD) is used.
- When fertility variation of the field moves in the one direction-Randomized block design (RBD) is used.
- When fertility variation moves in two directions- Latin square design (LSD) is used.
- When the number of treatments is sufficient by large and can form & square- Latin square design is used.
- When a very large number of germplasms is to be tested on a small piece of land- Augmented design would be a proper choice.

Basic principles of experimental designs: There are three basic principles of experimental designs, viz; replication, randomization and local control or error control. All these principles help in reducing the experimental error and thus make the experiment more efficient. These principles are briefly described below:

1. **Replication:** Repetition of treatments under investigation is known as replication. Increase in replication increases the precision by reducing the error to a great extent.
2. **Randomization:** The allocation of treatments to different plots by a random process is known as randomization of treatments. Randomization gives equal chance to all the treatments for being allotted to a more fertile plot as well as to a less fertile plot.
3. **Local control:** The principle of making use of greater homogeneity in groups of experimental units for reducing the experimental error is known as local control.

Completely Randomized Design (CRD): The design which is used when the experimental material is limited and homogenous is known as completely randomized design. This design is specially used for pot culture experiments. The important characteristics of this design are given below:

Layout: The whole field is divided into plots of similar shape and size. The number of plots is equal to the product of treatments and replications. These plots are then serially numbered.

Replications: There is no restriction on the number of replications in this design. The number of replications can vary from treatment to treatment. Normally, the number of replications for different treatments should be equal to get the estimates of treatments effects with same precision.

Randomization: The randomization is done treatment wise with the help of random table. First random numbers equal to the number of plots are taken from the random table. From these random numbers each treatment is

assigned numbers as per number of replications.

Local control: The principle of local control or error control is not adopted in this design. The error variance can only be reduced by selecting a homogeneous set of experimental units.

D	D	E	E	C
D	A	A	C	C
B	B	A	B	D
E	B	A	C	E

Layout of Completely Randomized Design (CRD) using 5 treatments and 4 replications.

ANOVA table for Completely Randomized Design (CRD).

Sources of variation	d.f.	S.S.	M.S.S.	S.S. / M.S.S.
Between treatments	(n-1)			
Within Treatments (error)	(N-n)			
Total	(N-1)			

Randomized Block Design (RBD): It is most commonly used experimental design in plant breeding and highly suitable for 20-25 entries when the fertility gradient of the experimental area moves in one direction. The main features of this design are:

Layout: Experimental field is divided in to homogeneous groups equal to the number of replications known as blocks. Then each block is further divided in to plots of similar shape and size equal to the number of treatments.

Replication: No restrictions on the number of replications. However, all the treatments should have equal number of replications.

Randomization: Treatments is allotted to the plots in each block by a random process. Separate randomization is used in each block.

Local control: The principle of local control is adopted in this design which control the experimental error by forming homogenous block.

6	7	2	9	3	8	1	10	4	5	Block-I
9	1	5	3	8	4	7	2	6	10	Block-II
2	10	8	5	9	1	4	6	3	7	Block-III
4	8	7	9	10	2	6	5	1	3	Block-IV

Layout of Randomized Block Design using 10 treatments and 4 replications.

ANOVA table for Randomized Block Design (RBD).

Sources of variation	d.f.	S.S.	M.S.S.	S.S. / M.S.S.
Blocks	(r-1)			
Varieties	(t-1)			
Error	(r-1) (t-1)			
Total	(rt-1)			

Latin Square Design (LSD): The experimental design which simultaneously controls the fertility variation in two directions is called Latin square design. The main features of this design are briefly described below:

Layout: The field is divided into homogeneous blocks is two ways. The blocks in one direction are commonly known as rows and in other direction as columns. The number of plots is same in each row and column which is equal to the number of treatments.

Replication: The number of replications is always equal to the number of treatments.

Randomization: The treatments are assigned in such a way that every treatment occurs once and only once in each rows and columns.

Local control: The principle of local control is adopted in this design which control the experimental error by forming rows and columns.

Rows	Columns				
	1	2	3	4	5
1	A	B	C	D	E
2	B	C	D	E	A
3	C	D	E	A	B
4	D	E	A	B	C
5	E	A	B	C	D

Layout of randomization in 5 x 5 Latin Square Design (LSD).

ANOVA table for Latin Square Design (LSD).

Source of variation	d.f.	S.S.	M.S.S.	F ratio observed
Rows	(n-1)			
Columns	(n-1)			
Treatments	(n-1)			
Error	(n-1) (n-2)			
Total	(n^2-1)			

Split Plot Design (SPD): The experimental design in which experimental plots are split or divided into main plots, sub-plots and ultimate plots is called split plot design. In this design several factors are studied simultaneously with different levels of precision. The factors are such that some of them require larger plots like irrigation, depth of ploughing and sowing dates, and others require smaller plots. The important characteristics of SPD are briefly described below:

Layout: The layout of this design consists of four steps as given below:

1. First the experimental field is divided into homogeneous blocks equal to the number of replications.
2. Then each block is divided into a number of plots equal to the number of levels of the first factor. These plots are known as main plots.
3. Then each main-plots is divided into a number of sup-plots equal to the number of levels of second factor, and
4. Then each sub-plot is divided into a number of ultimate plots equal to the number of levels of third factor.

Replications: There is no restriction on the number of replications unlike LSD. However, the number of replications should be uniform for all the treatments.

Randomization: The levels of the first factor are applied to the main plots of each block by the random process. The sub-plot treatments are then allotted at random to the sub-plots within each main plot. A separate randomization is carried out for each main plot. The levels of third factor are allotted to the ultimate plots in each sub-plots by a separate randomization.

Local control: The principle of local control is adopted in this design by forming homogeneous blocks.

<table>
<tr><td colspan="6">Block-1</td><td colspan="7">Block-2</td></tr>
<tr><td colspan="2" rowspan="2">Main Plot</td><td colspan="2" rowspan="2">Main Plot</td><td colspan="2" rowspan="2">Main Plot</td><td colspan="2">Sub-plot</td><td colspan="3">Sub-plot</td><td colspan="2" rowspan="2"></td></tr>
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<tr><td></td><td></td><td colspan="2">Ultimate</td><td colspan="2">plot</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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<tr><td colspan="6">Block-3</td><td colspan="7">Block-4</td></tr>
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Layout of Split Plot Design (SPD).

ANOVA table for Split Plot Design.

Source of variation	d.f.	S.S.	M.S.S.	F-ratio
Replication (r)	3			
Sowing dates (s)	2			
Error (a)	6			
Total	11			
Levels of Nitrogen (N)	3			
N x S	6			
Error (b)	27			
Total	47			
Seed rates (R)	1			
R x S	2			
R x N	3			
R x N x S	6			
Error (c)	36			
Total (RSNR-1)	95			

Lattice design: Lattice designs are incomplete block designs in which the number of varieties or treatments forms a square. The important characteristics of lattice design are given below:

Layout: The experimental field is divided into homogeneous parts equal to the number of replications. Each part is further divided into plots of equal size in such a way that the number of plots should form a square and each replication has equal plots in each direction, i.e. equal rows and columns.

Replications: There is no restriction on the number of replications. It would be better if the number of replications is 4 because it would make the layout compact.

Randomization: The treatments are randomized replication wise. Separate randomization in each replication.

Variation: The total variation is divided into five components, namely replications, rows, columns, treatments and error.

Replication-1					**Replication-2**				
11	21	9	13	5	6	13	22	23	15
17	2	23	25	3	19	25	20	4	12
4	14	6	16	19	24	8	2	9	17
18	22	24	1	7	11	18	14	16	1
8	12	20	10	15	21	3	10	5	7
18	9	17	23	11	25	4	14	18	9
6	1	25	8	24	7	12	11	8	24
14	22	5	15	20	23	6	2	22	15
3	13	16	2	12	13	20	16	10	3
21	19	70	10	4	1	17	5	19	21
Replication-3					**Replication-4**				

Layout of 5 x 5 Lattice Design using 4 replications.

ANOVA table for Lattice Design.

Source of variation	d.f.	S.S.	M.S.S.	F-ratio
Replications	(r-1)			
Rows	r(p-1)			
Columns	r(p-1)			
Treatments	(p^2-1)			
Error	(p-1) (pr-p-r-1)			
Total	(rp^2-1)			

Where, r = number of replications and p = square root of treatments.

Augmented design: The concept of augmented design was developed by Federer (1956). This is an experimental design which is used to test a large number of germplasm lines in a limited area. The important characteristics of this design are given below:

Layout: In this design, standard or check varieties are replicated among the cultures. Thus, standards are replicated, and cultures are non-replicated. The number of check varieties should be at least 4.

Replications: The number of replications depends on the number of check varieties to be used. For example, if we have 4 check varieties, we should have a minimum of 5 replications to get more than 10 error degrees of freedom.

Randomization: The check varieties are first randomly allocated to the plots of each block, and the germplasm lines are then randomly allocated to the remaining plots of each block.

Replications				
1	**2**	**3**	**4**	**5**
A	A	A	A	A
B	B	B	B	B
C	C	C	C	C
D	D	D	D	D
e	h	k	n	q
f	i	l	o	r
g	j	m	p	s

Layout of Augmented design.

Example: 8 entries of gram were put under a yield trial in RBD with four replications. Net plot size was 3.50 m x 1.80 m. give the plan of experiment and analyze the data.

Yield performance of gram varieties in four different replications.

Block-I/RI	3	4	8	7	5	1	2	6
Block-II/RII	5	6	3	2	1	8	4	7
Block-III/RIII	8	7	5	4	2	3	1	6
Block-IV/RIV	6	8	3	5	7	2	4	1

Plot yield data of gram varieties.

	Block/Replication				
Entries	**I**	**II**	**III**	**IV**	**Total**
1	43	93	80	90	306
2	125	118	93	61	397
3	130	95	95	126	446
4	126	119	107	108	460
5	140	123	127	105	495
6	162	103	130	130	525
7	121	124	110	112	467
8	133	117	100	118	468
Total	980	892	842	850	3,564

Correction factor (CF) = $(GT)^2$ / No. of observations (r x t) = $(3564)^2$ / 32

Total SS = $(43)^2 + (125)^2$ +----------+ $(118)^2$ – CF

= 413392 – 396940.5 = 16451.5.

Block SS = 1/8 [$(980)^2 + (892)^2$]+----------] - CF

= 398441 – 396940.5 = 1500.5

Treatment SS = ¼ [$(360)^2 + (397)^2$]+----------($(468)^2$] – CF

= 404881 – 396940.5

= 7940.5

Error SS = Total SS – Block SS – Treatment SS

= 16451.5 – 1500.5 – 7940.5

= 7010.5.

Analysis of variance (ANOVA) for plot yield in gram.

Source of variation	d.f.	S.S.	M.S.S.	F-value observed	F-table value at P 0.05
Blocks	3	1500.5	500.17	1.32	3.07
Treatments	7	7940.5	1134.36	3.00	2.49
Error	21	7010.5	378.12		
Total	**31**	**16451.5**			

Table value of F at 5 per cent is lower than the observed value. Hence, it is concluded that there was significant variation among all the eight tested entries of gram.

HARDY-WEINBERG LAW

In 1908, hardy-Weinberg independently discovered a principle concerned with the frequency of genes (alleles) in a population. In a random mating population, the frequencies of genes and genotypes remain constant generation after generation if evolutionary forces like selection, mutation, migration and random drift do not operate. For a single locus with two alleles (A and a), the gene frequencies remain in the ratio of p^2 (AA) : 2pq (Aa) : q^2 (aa)

Where, P and q are the frequencies of allele A and a, respectively. p + q are always equal to 1 or p = q = 0.50.

p + q = 1

p = 1 – q

q = 1 – p.

For a single locus with two alleles (A and a), the gene frequency from a sample is calculated as follows:

1. Proportion of A alleles = Number of A alleles / 2 x sample size
   Number of A alleles = 2 x AA genotypes + Aa genotypes.
2. Proportion of 'a' alleles = Number of a alleles / 2 x sample size
   Number of 'a' alleles = 2 x a genotypes + Aa genotypes.

Genotype frequency refers to the proportion of different genotypes in a mendelian population. It is also called zygotic frequency. For a single locus with two alleles (A and a), the genotypic frequency is calculated as follows:

1. Proportion of AA genotypes = Number of AA genotypes / sample size (total sample)
2. Proportion of Aa genotypes = Number of Aa genotypes / Sample size
3. Proportion of aa genotypes = Number of aa genotypes / sample size

Problem-1: In a sample of 100 plants of four o' clock plant, 40 were with red flower (RR), 30 with pink flower (Rr) and 30 with white flower. Find out the frequency of genes R and r.

Solution:

Given: Red flowered plants (RR) = 40
Pink flowered plants (Rr) = 30
White flowered plants (rr) = 30
Total = 100
Frequency of R alleles = 2 x 40 + 30 / 100 x 2 = 110 / 200 = 0.55
Frequency of r alleles = 2 x 30 + 30 / 100 x 2 = 90 / 200 = 0.45

Problem-2: In a random mating population of maize, the frequency of a dominant homozygote (RR) is 0.25. find out the frequency of heterozygotes and recessive (rr) individuals.

Solution: Given, frequency of RR genotypes = 0.25
P^2 (RR) = 0.25
$\therefore$ P = $\sqrt{0.25}$ = 0.5
q = 1 – p
$\therefore$ q = 1 – 0.5 = 0.5
$q^2 = (0.50)^2 = 0.25$
2pq = 2 (0.5 x 0.5) = 2 x 0.25 = 0.5
Frequency of heterozygotes (Rr) = 0.5
Frequency of recessives (rr) = 0.25
Frequency of dominant homozygotes (RR) = 0.25

HANDLING OF SEGREGATION GENERATIONS

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 notebook
12. Record of crosses
13. F1 generation
14. F2 segregation generation notebook.

Various types of record books maintained by a breeder

i. **Accession record:** A record is maintained of all the plant material received and tested at an experiment station. For a crop, the accession number starts with unity each year. This number is preceded by the year in which a strain is first planted. For example, if a variety/strain is received in the year 2016. number of the first entry may be 1601. Thus, the accession number helps in identifying a strain and indicates the year in which it was first tested in a trial. An accession record gives information about the accession number, name of a variety/strain, source of seed, seed source number, pedigree, brief botanical description and remarks. It will be mentioned as EC = Exotic collection IC = Indigenous collection.

ii. **Diary of crosses:** It gives a brief history of the crosses for reference. Each diary starts with purpose of attempting the cross. Then information about date of attempting the cross, number of female spikes/flower buds pollinated, number of hybrid seed obtained, number of hybrid seed sown, number of F_1 plants harvested, approximate number of F_2 seed obtained, number of selections made etc. is recorded.

iii. **Project book:** Each project on a breeding programme is numbered and a record of its name, year of start, purpose, breeding procedure followed and probable duration of the project is maintained.

iv. **Sowing plan:** A sowing plan of each trial is prepared at the time of sowing. The plan provides the information on field measurements, row numbers in a plot, placement of check plots, direction of sowing and name of entries in a replication. Date of sowing, number of replications, gross plot size and net plot size are recorded on the top of each plan

v. **Field notebooks:** These are used to record data on different characters relevant to each crop. These may be in the form of field registers or printed notebooks.