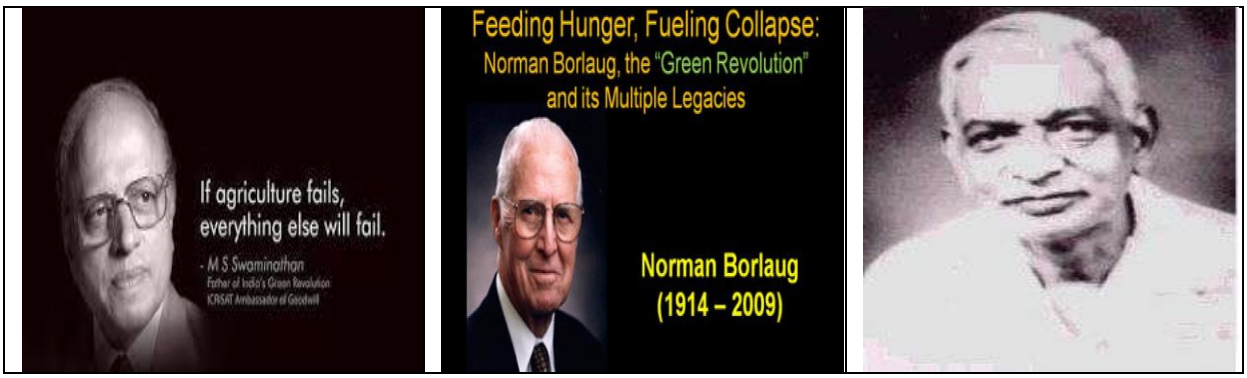
JUNAGADH AGRICULTURAL UNIVERSITY

PRACTICAL MANUAL
OF

FUNDAMENTALS OF PLANT BREEDING
(GPB 3.3)
THIRD SEMESTER B.Sc. (Hons.) Agriculture
(As per ICAR Fifth Dean Committee Recommendations)



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This is to certify that the practical work has been satisfactorily carried out by Mr. /Ms._____ In the course No. **GPB 3.3** with course title **"Fundamentals of Plant Breeding" (2+1)** of **Third Semester** B.Sc. (Hons.) Agriculture in the laboratory of Genetics and Plant Breeding, during the academic year _____ .

He/ She has certified _____ practical exercises out_____ in the subject of **Fundamentals of Plant Breeding**.

External Examiner **Course teacher**

Place:

Date:

Practical manual of

GPB 3.3- Fundamentals of Plant Breeding (2+1)

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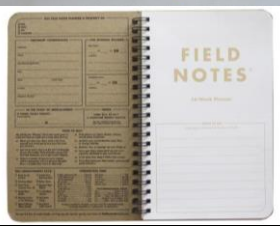
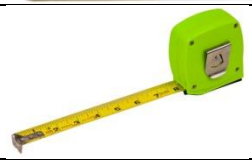
EXERCISE 1: PLANT BREEDER'S KIT **Date:**

Plant breeding is an art, science and technology of improving genetic makeup of plants for benefits of the human kind. For this, a plant breeder often requires artificial self-pollination, hybridization, evaluation of genotypes in the field. Following tools/equipments are required for emasculation, pollination and field experimentation for selection and filed observations.

Sr. No.	Tools/Equipments	Purpose/Use	
1	Fine pointed forceps	For emasculation, holding flowers and removing anthers	
2	Scissor	For cutting the vegetable parts and small/large size floral parts or flowers during hybridization	
3	Pointer/Needle	For incising the floral parts and removing the anthers during emasculation	
4	Alcohol or methylated spirit	A small vial is required to sterilize forceps, scissors, needles, brushes during hybridization programme	
5	Eye lens / Magnifying lens	To observe small flowers, stigmatic surface, dehiscence of anthers	
6	Automizer	It is used for spraying the gametocides during emasculation of flowers. e.g. 57% ethyl alcohol to kill Lucerne pollens	
7	Hair brush	For transferring the pollen grains without injuring to stigmas or pollens	

8	Parchment / Kite paper bag (white/red)	For protecting small size flowers during selfing and crossing	
9	Butter paper bag	For protecting the individual flowers or small twigs during selfing or crossing.	
10	Brown paper bag	To cover the panicles or large size influences during selfing and crossing	
11	Muslin cloth bag	To cover the whole plant (Chilli, Brinjal, Fennel) or twig (Mango) while selfing or crossing	
12	U-pins(u- clips)	For fastening the bags on earheads or flowers to keep the bag in proper position	
13	Soda straw tubes	For protecting the emasculated or pollinated flower buds and selfing	
14	Wire ring/ Smooth threads	For tying closed buds for selfing. They are inserted / tied in axis of flowers to identify selfed/crossed flower.	

15	Small white tag	For identifying the flower or a small twig during hybridization and writing detailed information about crossing with pencil and then inserted on pedicel or peduncle	
16	Pencil	For writing field labels or field bags as it will not erase or spread during rains, dew or under intense light	
17	Aluminum label with wire	For tagging the flowers in fruit crops or tree species after crossing. It is also used for identification of selected trees	
18	Luggage labels (white or yellow)	For tagging the large sized plants while rouging or during selection	
19	Waxy threads	For fastening the luggage labels on plants	
20	Sample bag (Yellow)	For storing the crossed seeds in small quantity	
21	Field note books	To note down all breeding activities and daily observation in the field regarding germination, flowering, morphological, description, date of emasculation, pollination, number of cross attempted and seeds setting	
22	Measure scale	To measure plant height and prepare field layout.	

Exercise:

A. List the different tools/equipments of breeder's kit and write down its uses.

EXERCISE 2: STUDY OF GERMPLASM OF VARIOUS CROPS Date:

Germplasm: The sum total of hereditary material, i.e., all the alleles of various genes, present in a crop species and its wild relatives.

Genetic resources: The sum total of genes in a crop species.

Germplasm is the basic material with which a plant breeder has to initiate his breeding programme.

Important features of plant genetic resources are

1. Gene pool represents the entire genetic variability or diversity available in a crop species.
2. Germplasm consists of land races, modern cultivars, obsolete cultivars, breeding stocks, wild forms and wild species of cultivated crops.
3. Germplasm includes cultivated species, wild species and relatives of crop plants.
4. Germplasm is collected from the centres of diversity, gene banks, gene sanctuaries, farmer's fields, markets and seed companies.
5. Germplasm is the basic material for launching a crop improvement programme.
6. Germplasm may be indigenous (collected within country) or exotic (collected from foreign countries).

Germplasm consists of the following five types of materials:

1. **Land Races**
   - These are primitive varieties, which had evolved without a systematic and sustained plant breeding effort.
   - They are sources of many valuable genes including those for adaptation. They are storehouses of genetic variability.
   - Adapted to the local soil type, climatic conditions etc.
2. **Obsolete Varieties**
   - These varieties were developed by systematic breeding effort, were once commercially cultivated but are no more grown.
   - They have some desirable features.
   - For example, wheat varieties K 65, K 68, Ph 591, many NP series varieties etc. are obsolete varieties.
3. **Varieties in Cultivation**
   - The varieties in cultivation are the easiest to use in breeding programmes.
   - They form a major part of a working collection.
   - They are good sources of genes for yield, quality etc.
   - They can be introduced in a new area, and directly released for cultivation.

4. **Breeding Lines**
   - These are lines/populations developed in breeding programmes.
   - They often contain valuable gene combinations.
   - This includes nearly homozygous lines, mutant lines, lines derived from biotechnology programmes and, now, transgenic lines.
5. **Wild Forms**
   - *Wild forms* are the wild species from which crop species were directly derived.
   - They are easy to cross with the concerned crop species.
6. **Wild Relatives**
   - *Wild relatives* include all other species, which are related to the crop species by descent during their evolution.
   - Wild relatives are much more difficult to hybridize with crops than are the wild forms.
7. **Mutants**
   - Mutants can be identified in nature as well as can be induced through the use of physical and chemical mutagens.
   - Mutation breeding is non-conventional method which is used when the variability for desired character is not found in the genetic material of cultivated species and its wild relatives.

Genetic resources can be broadly grouped into two types, depending on the state of their domestication as (1) cultivated germplasm and (2) wild germplasm.

Alternatively, they may be termed as (1) indigenous (from the country in question) and (2) exotic (from another country) based on their place of origin.

GENE POOL CONCEPT

***Gene pool*: All the genes and their alleles present in the individuals, which hybridize or can hybridize with each other.**

In some sense, gene pool describes a concept similar to germplasm.

The gene pool is classified into three groups:

(1) Primary gene pool (GP_1),
(2) Secondary gene pool (GP_2)
(3) Tertiary gene pool (GP_3)

1. Primary Gene Pool (GP_1)

- It includes all such strains and species, which hybridize readily with each other and give rise to fertile hybrids.
- It consists of all the different strains or varieties of a crop species and some related species.
- The members of primary gene pool are the most commonly used in breeding programmes.

2. Secondary Gene Pool (GP_2)

- Members of secondary gene pool are all those species that hybridize with the members of primary gene pool with some to considerable difficulty and the hybrids are partially fertile.
- These species are difficult to hybridize with those of GP_1 due to ploidy differences, chromosome alterations or genetic barriers.
- Gene transfers from GP_2 to GP_1 are possible but usually difficult.
- Members of this group are often used in breeding programmes.

3. Tertiary Gene Pool (GP_3)

- The species belonging to this group cross with the members of primary gene pool with considerable to great difficulty, and hybrids, if produced, are anomalous, lethal or completely sterile.
- Gene transfers from this group to the primary gene pool are very difficult and require special techniques.
- Hybrids are invariably sterile.
- Gene transfers from GP_3 to GP_2 are relatively easier.
- GP_3 is used only occasionally in breeding programmes.

GENETIC EROSION

***Genetic erosion*: The gradual loss of variability from cultivated species, and their wild forms and wild relatives.**

- Genetic erosion is a creation of man since his success in plant breeding is the chief cause of genetic erosion.

The main causes of genetic erosion are briefly summarized below.

- Replacement of genetically variable land races ('Desi' varieties) by the improved, genetically uniform pureline or hybrid varieties.
- Improved crop management practices have virtually eliminated the weedy forms of many crops.
- Increasing human needs have extended farming and grazing into forests, the habitats of most wild species. This has led to the extinction of many wild relatives of crops.
- Developmental activities like hydroelectric projects, roads, industrial areas, railways, buildings etc. have also disturbed the wild habitat. Often wild relatives of crops are destroyed due to these activities.
- ***Ideal, solution to the problem of genetic erosion is collection and conservation of the germplasm of cultivated plant species.***

***Germplasm regeneration*:** Growing seeds of the various entries in field and harvesting fresh seeds for further storage. Germplasm collections have to be re-generated after every few years since seeds of most species lose viability during storage.

ACTIVITIES IN GERM PLASM CONSERVATION

- The various activities in germplasm conservation can be grouped into the following categories:
  (1) Collection of germplasm
  (2) Conservation
  (3) Evaluation
  (4) Cataloguing, *i.e.,* data storage and retrieval,
  (5) Multiplication and distribution, and
  (6) Utilization.

In addition, at world level International Plant Genetic Resources Institute **(IPGRI),** Rome looks after the *Training of personnel* and *Global coordination*.

COLLECTION OF GERMPLASM

- The process of obtaining the various germplasm accessions for a germplasm collection is known **as *collection of germplasm***.

This can be done in two chief ways

1. Exploration and Collection

- Trips for collection of various forms of crop plants and their related species are termed as explorations.
- *Explorations are the primary source of all the germplasm present in various germplasm collections.*
- Therefore, cultivated forms like land races, open-pollinated varieties etc., wild forms and wild relatives are all collected.

2. Procurement from other Agencies

- Germplasm can be obtained from other agencies concerned with germplasm conservation, from research institutions, individuals or companies.

GERMPLASM CONSERVATION

Conservation refers to protection of genetic diversity of crop plants from genetic erosion.
The germplasm has to be maintained in such a state that there is minimum risk for its loss is called *Germplasm conservation.*

Germplasm can be conserved either

A. ***In situ Germplasm Conservation***

- Conservation of germplasm in its natural habitat or in the area where it grows naturally.
- This is achieved by protecting this area from human interference. This area is called *natural park, biosphere reserve* or *gene sanctuary.*
- A gene sanctuary is best located within the Centre of origin of crop species concerned, preferably covering the micro-centre within the Centre of origin.
- NBPGR, New Delhi, is making attempts to establish gene sanctuaries in Meghalaya for *Citrus* and in the North-Eastern region for *Musa, Citrus, Oryza, Saccharum* and *Mangifera.*

B. Ex situ Germplasm Conservation

- Conservation of germplasm away from its natural habitat.

This method has following three advantages:

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

It can be achieved in the following 5 ways:

(1) Seed banks
(2) Plant or field banks
(3) Shoot tip banks
(4) Cell and organ banks
(5) DNA banks

1. Seed Banks

- In seed banks germplasm is stored as seeds of various accessions. Virtually all gene banks are essentially seed banks. Seed conservation is quite easy, relatively safe and ordinarily needs minimum space. Under suitable conditions, seeds of many species can be stored for upto 50-100 years**.**
- Seeds are classified, mainly on the basis of their storability, into two major groups:

a. *Orthodox Seeds.*

- Seeds of this type can be dried to moisture content of 5% or lower without lowering their viability.
- Most crop seeds belong to this category.
- Such seeds can be easily stored for long periods.

b. *Recalcitrant Seeds.*

- The viability of seeds drops drastically if their moisture content is reduced below 12-30%.
- Seeds of many forest and fruit trees, and of several tropical crops like *Citrus*, cocoa, coffee, rubber, oil palm, mango, jack fruit etc. belong to this group.

- Such seeds present considerable difficulties in storage.
- Therefore, germplasm of such plants are conserved by alternative approaches (Field gene bank).

2. Plant Banks

- A *plant bank* is an orchard or a field in which accessions of fruit trees or vegetatively propagated crops are grown and maintained.

  Limitations

  - Requires large areas
  - Expensive to establish and maintain
  - Prone to damage from disease, insects, man-made or natural disasters abd human handling errors.

3. Shoot Tip Banks

- In such gene banks, germplasm is conserved as slow growth cultures of shoot-tips and node segments.

  Benefits

  - Conservation free from disease and pests
  - Used for seeds which either do not produce viable seeds or produce recalcitrant seeds
  - Consists of sub-culturing the cultures, which may be done every 6 months to 3 years and every time it requires short period of time for sub-culturing

4. Cell and Organ Banks

- A germplasm collection based on Cryo-preserved (at -196°C in liquid nitrogen) embryogenic cell cultures, shoot-tips and or somatic/zygotic embryos may be called *cell and organ bank.*

5. DNA Banks

- In these banks, DNA segments from the genomes of germplasm accessions are maintained as cosmid clones, phage lysates or pure DNA (For relatively short periods).
- These DNA segments can be evaluated and the desired ones may be used to produce transgenic plants.
- Applicable for conservation of genetic materials of extinct species since DNA extracted from well preserved herbarium specimens can often be cloned.
- However, it is very expensive and highly sophisticated. A world-wide network of DNA banks for threatened/endangered species has been established.

GERMPLASM EVALUATION

- *Evaluation* consists of assessment of the germplasm accessions for their various features or traits of some known or potential use in breeding programmes.

- Generally, germplasm accessions are evaluated for morphological, physiological, biochemical, plant pathological *(i.e.*, disease resistance), entomological *(i.e.*, insect, resistance) and other features.
- IPGRI, Rome has developed model lists of descriptors (= characters) for which germplasm accessions of various crops should be evaluated.

GERMPLASM CATALOGUING, DATA STORAGE AND RETRIEVAL

- Each germplasm accession is given an accession number.
- This number is prefixed, in India, with 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 features or descriptors is also recorded.
- Therefore, catalogues of the germplasm collections for various crops are published by the gene banks.

GERMPLASM MULTIPLICATION AND DISTRIBUTION

- The germplasm accessions requested by breeders/researchers are multiplied and supplied to them, usually without cost.
- Active collections are used for this purpose.

GERMPLASM UTILISATION

- The germplasm can be used in a breeding programme in the following 3 ways:
  (1) Direct release as a variety,
  (2) It may be subjected to selection for developing a variety and
  (3) It may be used as parents in hybridization programmes.

NBPGR:

- National Bureau of Plant Genetic Resources was established by ICAR in 1976. in New Delhi
- The basic function of NBPGR is to conduct research and promote collection, conservation, evaluation, documentation and utilization of crop genetic resources in India.

Types of seed collections

Based on the use and duration of conservation, seed collections are of three types

1. Base collections
2. Active collections
3. Working collections

- **Base collections: It is also known as principal collection.**
- **These consist of all the** accessions present in the germplasm of a crop. They are stored at about -180C or -200C with 5 + 1% moisture content; they are disturbed only for regeneration.
- When the germination of an accession falls below, usually, 95% of its germination at the start of storage, the accession is regenerated.

For reasons of safety, duplicates of base collections should be conserved in other germplasm banks as well. High quality orthodox seeds can maintain good viability upto 100 years

2. Active collections:

- **The accessions in an active collection are stored at temperatures** below 150C (often near 00C), and the seed moisture is kept at 5%.
- The storage is for medium duration, i.e., **10-15 years**. These collections are actively utilized in breeding programme.
- These collections are used for evaluation, multiplication and distribution of the accessions. They are usually maintained by multiplying the seeds of their own accessions. But from time to time, base collection material should be used for regeneration of these collections.
- Germination test is carried out after every 5-10 years to assess the reduction in seed viability.

3. Working collections: The accessions being actively used in crop improvement programmes constitute working collection.

Their seeds are stored for **3-5 years** at less than 150C and they usually contain about 10% moisture. These collections are maintained by the breeders using them.

❖ **Core collection**

The concept of core collection was proposed by Franked it refers to a subset of base collection which represents the large collection. Or a limited set of accessions derived from an existing germplasm collections.

Exercise:

1. Define: Germplasm, Exploration, Recalcitrant seeds, Genetic erosion, Exotic collections
2. Enlist the various forms of germplasm
3. Write down about gene pool concept and its type
4. Explain activities in germplasm conservation
5. Difference the followings.
    a. Orthodox Seeds **and** Recalcitrant Seeds
    b. In situ **and** Ex situ Germplasm Conservation
    c. GP_1 **and** GP_2

EXERCISE 3: STUDY OF MODE OF POLLINATION AND LIFE CYCLE OF AN ANGIOSPERMIC PLANT Date:

Flower: A flower is highly metamorphosed shoot meant essentially for reproduction in the plants.

Pollination: Pollination refers to the transfer of pollen grains from anthers to stigmas.

Pollen from an anther may fall on to the stigma of the same flower leading to **self-pollination or autogamy**. When pollen from flowers of one plant is transmitted to the stigmas of flowers of another plant, it is known as **cross-pollination or allogamy.**

A third situation, **geitonogamy**, results when pollen from a flower of one plant falls on the stigmas of other flowers of the same plant, e.g., Maize. The genetic consequences of geitonogamy are the same as those of autogamy.

Self-pollination

- Believed to have originated from cross-pollinated ancestors.
- Must have hermaphrodite flowers.
- 100% self -pollination is not occur & cross -pollination may occur up to 5%.
- The degree of cross-pollination in self-pollinated species is affected by several factors, e.g., variety environmental conditions like temperature and humidity and location.

Mechanisms of self-pollination

1. **Bisexuality:** Presence of male and female organs in the same flower.
2. **Homogamy:** Maturation of anther and stigma of a flower at the same time
3. **Cleistogam**y: In this case, flowers do not open at all. This ensures complete self-pollination since foreign pollen cannot reach the stigma of a closed flower. Occurs in some varieties of wheat, oats, and barley.
4. **Chasmogamy:** In some species, the flowers open, but only after pollination has taken place. This occurs in many cereals, such as, wheat, barley, rice and oats. Since the flower does open, some cross-pollination may occur.
5. **Position of anther:** In crops like tomato and brinjal, the stigmas are closely surrounded by anthers. Pollination generally occurs after the flowers open. But the position of anthers in relation to stigmas ensures self-pollination.
6. In some species, flowers open but the stamens and the sigma are hidden by other floral organs. In several legumes, e.g., pea, mung, urdbean, soybean and gram. The stamens and the stigma are enclosed by the two petals forming a keel.
7. In a few species, stigmas become receptive and elongate through staminal columns. This ensures predominant self -pollination.

Genetic Consequences of autogamy

- Leads to a very rapid increase in homozygosity. Therefore, populations are highly homozygous.

- Do not show inbreeding depression, but may exhibit considerable heterosis. Therefore, the aim of breeding methods generally is to develop homozygous varieties.

FEATURES OF AUTOGAMY

- Homozygous and have advantage of homozygosity
- No recessive deleterious genes
- Homozygous balance and show no inbreeding depression
- New gene combination are not possible due to regular self-pollination
- Inbreeders have generally narrow adaptation and are less flexible

List of self-pollinated crops

Cereals	**Legumes**	**Vegetables**	**Oilseeds**
Wheat	Pea	Tomato	Sesamum
Rice	Cowpea	Chilli	Linseed
Barley	Chickpea	Brinjal	Groundnut
Oat	Mungbean	Potato	Soybean

Cross Pollination

- In cross-pollinating species, the transfer of pollen from a flower to the stigmas of the others plant.
- Many of the crop plants are naturally cross-pollinated. In many species, a small amount (up to 5-10 %) of selfing may occur.

Mechanisms promoting allogamy

1. **Dicliny or unisexuality**: Flowers are either staminate (male) or pistillate (female).
   i. **Monoecy.** Staminate and pistillate flowers occur in the **same plant, either in the same inflorescence**, e.g., Castor, mango and coconut, or in separate inflorescences, maize, chestnut, strawberries, rubber, grapes and cassava.
   ii. **Dioecy.** The male and female flowers are **present on different plants,** i.e., the plants in such species are either male or female, e.g., papaya, date palm, pointed gourd, hemp, asparagus, and spinach. In general, the sex is governed by a single gene, e.g., asparagus and papaya.

   In some cases, there are hermaphrodite plants in addition to males and females, and a number of intermediate forms may also occur.
2. **Dichogamy**: Stamens and pistils of hermaphrodite flowers may **mature at different times** facilitating cross pollination.
   i. **Protogyny:** Pistils mature before stamens e.g. Pearl millet (Bajra).
   ii. **Protandry:** Stamens mature before pistils e.g. Maize and sugar beets
3. **Herkogamy (Prevention by physical barrier):** In Lucerne or alfalfa, **stigmas are covered with a waxy** film. The stigma does not become receptive until this waxy film

is broken. The waxy membrane is broken by the visit of honey bees which also effect cross-pollination.

4. **A combination of two or more of the above mechanisms** may occur in some specie. This improves the efficiency of the system in promoting cross-pollination. e.g. Maize: Monoecy and Protandry.
5. **Self-Incompatibility:** It refers to the failure of pollen to fertilize the stigma of same flower or other flowers on the same plant.
    - Sporophytic self-incompatibility: Controlled by the genotype of the pollen producing plant.
    - Gametophytic self-incompatibility: Controlled by the genotype of the pollen itself.
6. **Male Sterility:** Male sterility refers to the absence of functional pollen grains in hermaphrodite flowers.
    - Genetic male sterility : Controlled by nuclear genes
    - Cytoplasmic male sterility : Controlled by cytoplasmic genes
    - Cytoplasmic- Genetic male sterility : Controlled by both nuclear and cytoplasmic genes
7. **Heterostyly:** Style and filaments in a flower are of a different length e.g. Linseed

Genetic consequences of allogamy

- Preserves and promotes heterozygosity in a population.
- Show mild to severe inbreeding depression and a considerable amount of heterosis.
- The breeding methods aim at improving the crop species without reducing heterozygosity to an appreciable degree.
- Hybrid or Synthetic varieties are the aim of breeder wherever the seed production of such varieties is economically feasible.

FEATURES OF ALLOGAMY

- Random matting
- Heterozygous & advantage of heterozygosity
- Contains some deleterious recessive genes
- Show considerable inbreeding depression
- Permits new gene combinations from different sources.
- Variability is distributed over entire population.
- Wide adaptability and more flexibility to environmental changes.

List of cross pollinated crops

Cereals	**Vegetables**	**Oilseeds**	**Forages**	**Fruits**
Maize	Bitter gourd	Castor	Lucerne/Alfalfa	Banana
Bajra	Onion	Sunflower	Napier grass	Mango
Rye	Cabbage	Oil palm	Sudan grass	Aonla

Often cross pollinated species

- Cross-pollination often exceeds 5 per cent and may reach 30 -50 per cent. e.g., Sorghum, Cotton, Okra, Pigeon pea
- The genetic architecture of such crops is intermediate between self-pollinated and cross-pollinated species.
- Consequently, in such species breeding methods suitable for both of them may be profitably applied.
- Hybrids are superior to others.

List of often cross pollinated crops

Cereals	**Pulses**	**Fibres**	**Others**
Sorghum	Pigeon pea	Cotton	Tobacco

Pollination syndrome

Flower characteristics or traits which attracts particular type of pollinators.

- Combination of colour
- Odour
- Quantity of nectar
- Location and type of pollen and flower structure

Rewards for pollinators

- Nectar
- Pollen
- Shelter
- Heat

Types

1) Abiotic pollination syndromes

- Wind pollination (Anemophily)
- Water pollination (Hydrophily)

2) Biotic pollination syndromes

- Bee pollination (Melittophily)
- Wasp pollination
- Butterfly pollination (Psychophily)
- Moth pollination (Phalaenophily)
- Fly pollination (Myophily and Sapromyophily)
- Bird pollination (Ornithophily)
- Bat pollination (Chiropterophily)
- Beetle pollination (Cantharophily)

DETERMINATION OF MODE OF POLLINATION

- The first step in determining the mode of pollination of a species is to critically examine its flowers.
- Mechanisms like dioecy, monoecy, protogyny, protandry and cleistogamy are easily detected. They clearly indicated the mode of pollination.
- The second step consists of isolating single plants and recording seed set under isolation. Space isolation, i.e. individual plants grown at sufficient distance to prevent cross-pollination, is preferable to isolation by bags or cages since the latter may create an environment unfavourable for pollination and seed set.
- Failure to set seed in isolation proves the species to be cross-pollinated. However, setting of seeds is only indicative of self-pollination.
- Finally, the effects of selling (inbreeding) on the vigour of plants should be studied. Loss in vigour due to inbreeding is common in cross-pollinators, but self-pollinators show no inbreeding depression.

DETERMINATION OF AMOUNT OF CROSS-POLLINATION

- The amount of cross-pollination is determined by planting two strains of the concerned species in a mixed stand.
- One strain is homozygous for a dominant character, preferably an easily recognizable seed or seedling character, while the other strain has the recessive form of the character.
- The two strains arc planted in such a manner that each plant of the recessive strain is surrounded by plants of the dominant strain to provide abundant pollen.
- Seeds from the plants of only recessive strain are harvested. The percentage of these seeds carrying the dominant allele represents the percentage of cross-pollination in the species.
- The frequency of cross-pollination varies greatly with the variety, weather conditions and location.
- For example, in a study on safflower the estimates of out crossing in different varieties grown in the same year at the same location ranged from 0-8.7 per cent.
- Similarly, the amount of cross-pollination in a single variety grown at several locations varied from 1.3 to 9.8 per cent.
- Therefore, such a study should include several varieties of the crop and the study should be conducted at several locations for two or more years.

The life cycle of an angiospermic plant describe by following phases:

1. Production of spores and gametes by the action of sporogenesis and gametogenesis.
2. Pollination
3. Fertilization
4. Formation of zygote followed by seed & fruit from ovule & embryo sac (ovary), respectively.
5. Germination of plant from seed.

Fertilization: It is the fusion of male and female gametes.

Gametes: Sexual reproductive unit which is produced by sporogenesis and gametogenesis.

Process of fertilization:

- After pollination the pollen grain germinates on the stigma and pollen tube elongates through the style and enters the ovary to ovule through the microphyl.
- At this time, function of vegetative nucleus is over and it degenerates.
- Pollen after reaching at ovule enters one of the synergids.
- Tips of pollen tube dissolved enzymatically and one of the male gamete fuse with egg cell to from the zygote (2n), which form a protective wall around itself to form the oospore.
- The second male gamete fuses with the two polar nuclei which give rise to triploid endosperm (3n) nucleus (This is called double fertilization).
- Two synergids degenerates immediately after fertilization and three antipodals may degenerate even before fertilization.
- The oospore (2n) give rise to the embryo and the triploid endosperm nucleus give rise to the endosperm (3n) and this is known as triple fusion.
- The ovule and ovary gives rise to seed and fruit respectively (Figure 3.1)

Exercise:

1. Define: Homogamy, Heterostyly, Double fertilization, Triple fusion
2. Explain mechanisms for self and cross pollination in plants
3. Enlist the types of pollination syndrome
4. Explain determination of mode of pollination
5. Describe in brief about life cycle of an angiospermic plant
6. Write down about determination of the amount of cross-pollination

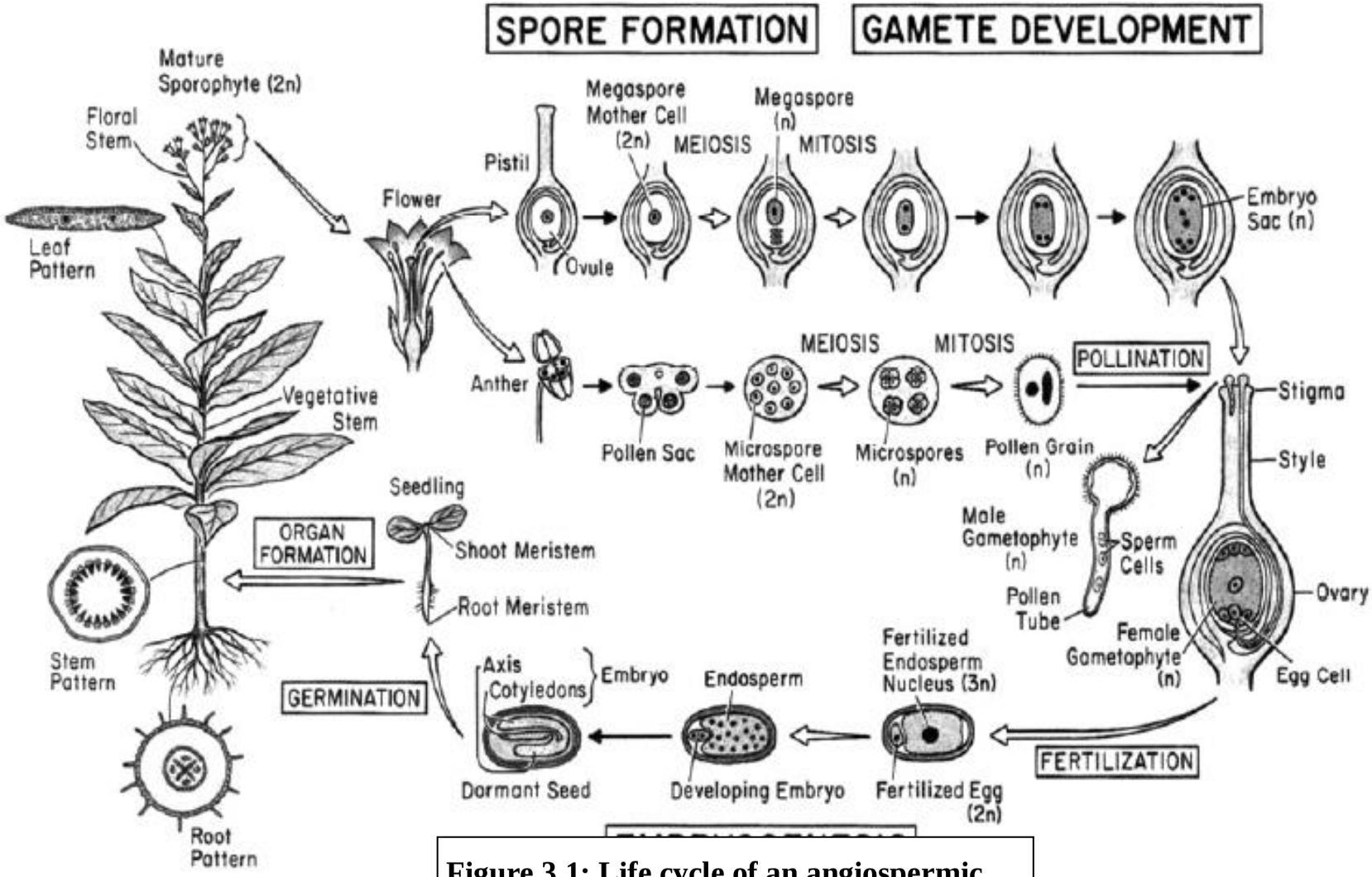


Figure 3.1: Life cycle of an angiospermic

EXERCISE 4: CONSEQUENCES OF INBREEDING ON GENETIC STRUCTURE OF RESULTING POPULATION

Date:

Cross-pollinated species and asexually reproducing species are naturally highly heterozygous. They generally show reduction in vigour and fertility due to inbreeding, while, hybridization involving unrelated strains often leads to increased vigour and fertility. These phenomena are of great significance in breeding of these crops as a breeding scheme must keep inbreeding to the minimum.

❖ INBREEDING DEPRESSION

- Mating between individuals related by descent is called **inbreeding.**
- Degree of inbreeding may be higher for more closely related the individuals in the population on selfing.
- The degree of inbreeding of an individual is expressed as inbreeding coefficient (F). The value of F for an individual is the probability of the two alleles of a gene present in an individual to have been derived from a single allele of a common ancestor, i.e., an ancestor that occurs in the pedigree of both maternal and paternal parents of this individual.
- In a random mating population, the value of F for any individual is 0, while that for an individual produced by selfing of a plant from a random mating population is 1/2. The value of F is cumulative over generations.
- The chief effect of inbreeding is an increase in homozygosity proportionate to the degree of inbreeding. Cross-pollinated and vegetatively reproducing species show reduced vigour and fertility upon selfing. This is termed **as inbreeding depression.**
- In 1876, Darwin concluded that progeny obtained from self-fertilization were weaker than those derived from outcrossing. Detailed information on inbreeding depression in Maize was given by East (1908) and Shull (1909).

❖ EFFECTS OF INBREEDING

- Appearance of lethal and sublethal traits (=alleles), e.g., chlorophyll deficiency, etc. Plants showing such traits usually can't survive in nature.
- A general reduction in vigour and plant size.
- A rapid reduction in rate of reproduction and reproductive ability of an individual.
- The population rapidly separates into phenotypically distinct lines due to increased homozygosity (7 selfing generations leads to >99% homozygosity) and random fixation of alleles.
- A drastic decline in yield is a common feature. An inbred is a homozygous line in cross-pollinated crop produced and maintained by close inbreeding. In maize, performance of inbreds isolated from open-pollinated populations yielded 50% of its parental varieties.

DEGREES OF INBREEDING DEPRESSION

The degree of inbreeding depression depends on the plant species concerned. But within a species, the extent of depression is related to the value of F and the relative fitness of the trait in question. Inbreeding depression is common in the case of traits that form an important component of fitness. Those traits that contribute little to fitness, usually show little or no inbreeding depression. The extent of inbreeding depression observed in various plant species may be grouped into the following four categories:

1. **High Inbreeding Depression**: A large proportion of plants produced by selfing does not survive. e.g. Alfalfa, Carrot. Loss in vigour and fertility is so high that only few lines can be maintained after 3-4 generations of inbreeding. The yields of surviving inbred lines are usually <25% of that of the parent open-pollinated varieties.
2. **Moderate Inbreeding Depression:** In species like Maize, jowar (Sorghum), Bajra etc., a large proportion of plants can be maintained under self-pollination. Many lines may be lost due to reduced fertility. Inbred lines may yield as much as 50% of the parent open-pollinated varieties.
3. **Low Inbreeding Depression:** In species like onion, many cucurbits, sunflower, hemp, etc. only a small proportion of plants show lethal or sublethal traits. Some rare lines can't be maintained due to poor fertility. There is little or no reduction in yield and some inbred lines may yield as much as the parent open-pollinated varieties.
4. **Zero - No Inbreeding Depression:** Populations of self-pollinated crops do not show any inbreeding depression. However, when the F1's from various crosses are selfed, a variable degree of inbreeding depression is observed in the F2 generation; this is estimated as follows.

Inbreeding depression (%) = $\frac{\overline{F_1}-\overline{F_2}}{\overline{F_1}}$ X 100

Where,

$\overline{F1}$ = Mean performance of F_1 generation

$\overline{F2}$ = Mean performance of F_2 generation

Formula for calculating homozygosity and heterozygosity

Homozygosity (%) (AA or aa) = $[2^n\text{-}1/2^n]^m$ x 100

Heterozygosity (%) (Aa) = $[1/2^n]^m$ x 100

Where, n = Number of selfing generations.

m = Number of gene pairs segregating.

This also gives the degree of homozygosity with respect to any number of genes.

Table 4.1: Effect of selfing on the frequency of homozygosity and heterozygosity with respect of a single locus Aa.

No. of generation of selfing	Frequency (%)			Frequency (%)	
	AA	Aa	aa	Homozygosity	Heterozygosity
S_0	-	100	-	-	100
S_1	25	50	25	50	50
S_2	37.5	25	37.5	75	25
S_3	43.75	12.5	43.75	87.5	12.5
S_4	46.875	6.25	46.875	93.75	6.25
S_5	48.437	3.125	48.437	96.874	3.125
S_6	49.218	1.562	49.218	98.436	1.562
S_7	49.608	0.781	49.508	99.216	0.781

Exercise:

1. Define: Inbreeding, Inbreeding depression
2. Explain the various categories of extent of Inbreeding depression
3. Calculate homozygosity (%) and heterozygosity (%) in population having eight generations of selfing for two independently segregating gene pairs.

EXERCISE 5: ESTIMATION OF HETEROSIS AND INBREEDING DEPRESSION

Date:

- The superiority of F_1 hybrids in one or more characters over its parents is termed as **heterosis.** The term hybrid vigour is used as synonym for heterosis.
- It is also defined as increase in fitness and yield over its parental values.
- The three main causes of heterosis are over dominance, dominance and epistasis, of these dominance theory is the widely accepted one.
- **The term heterosis was given by Shull in 1914**

ESTIMATION OF HETEROSIS: Heterosis is estimated in three different ways

1. **Average heterosis/Relative heterosis/ Mid-parent heterosis :**
   - When the heterosis is estimated over the mid parental value (i.e. mean value or average of the two parents involve in hybrid (F_1).

   Relative heterosis (RH) (%) $= \frac{\overline{F1} - \overline{MP}}{\overline{MP}} \times 100$

   $\overline{MP} = \frac{\overline{P1} + \overline{P2}}{2}$

2. **Heterobeltiosis/ Better parent heterosis (Fonseca and Patterson, 1968):**
   - When the heterosis is estimated over the superior or better parent.

   Heterobeltiosis (HB) (%) $= \frac{\overline{F1} - \overline{BP}}{\overline{BP}} \times 100$

Where, $\overline{BP}$ = mean value (over replications) of the better parent of the particular cross.

3. **Standard /Economic /Useful heterosis (Meredith and Bridge, 1972):**
   - The term useful heterosis was used by Meredith and Bridge (1972).
   - It refers to the superiority of F_1 over the standard commercial check variety or hybrid.
   - This type of heterosis provides direct practical value in plant breeding.

   Standard heterosis (SH) (%) $= \frac{\overline{F1} - \overline{SC}}{\overline{SC}} \times 100$

Where, $\overline{SC}$ = mean value (over replications) of the standard check.

Test of Significance for Heterosis

Standard errors (S.E) and critical differences (C.D.) for heterosis, heterobeltiosis and standard heterosis were calculated by using following formulae.

S.E. (M.P.) = $\sqrt{\frac{3M_e}{2r}}$

C.D. (M.P.) = S.E. $_{(MP)}$ × table $t_{0.05}$ and $t_{0.01}$ at error d.f.

S.E. (B.P./S.C.) = $\sqrt{\frac{2M_e}{r}}$

C.D. (**B.P./S.C.**) = S.E. $_{(BP/SC)}$ × table $t_{0.05}$ and $t_{0.01}$ at error d.f.

Where,

r = Number of replications

M_e = Error mean square

t = Table value of 't' at error degree of freedom corresponding to 5 per cent or 1 per cent level of significance.

Alternatively, significance of heterosis value was tested using 't' test.

$$\textit{Calculated } t = \frac{\overline{F1} - \overline{MP} \text{ or } \overline{BP} \text{ or } \overline{SC}}{\text{S.E. of heterosis over } \overline{MP} \text{ or } \overline{BP} \text{ or } \overline{SC}}$$

Calculated t values were compared with tabulated **'t'** values at error degree of freedom for test of significance.

Conclusions:

- If table $t_{0.05,}$ error df is higher than calculated 't' value then result is non-significant.
- If calculated 't' is higher than table $t_{0.05,}$ error df value then result is significant (symbolized as '*'), but if calculated 't' is higher than table $t_{0.01}$, error df value then result is highly significant (symbolized as '**').

INBREEDING DEPRESSION:

- Inbreeding depression: reduction or loss in vigour and fertility as a result of inbreeding.
- Since the maximum decline is reflected in F_2 generation, the inbreeding depression can be computed by relative data on F_1 and F_2 for any character as under :

Inbreeding Depression (%) = $\frac{\overline{F_1} - \overline{F_2}}{\overline{F_1}}$ X 100

Where,

$\overline{F1}$ = Mean performance of F_1 generation

$\overline{F2}$ = Mean performance of F_2 generation

Exercise:

A total of 45 maize hybrids and their 10 inbred parents were evaluated in a Randomized Complete Block Design (RCBD) with three replications. The following is the data on grain yield per plant (grams). Work out the different types of heterosis per cent basis and its test of significance. Comment on the results. Assume **HIM 129** as standard check.

Genotype	R I	R II	R III	Genotype	R I	R II	R III
IL112 × IL113	122.7	114.2	127.1	IL101 × IL109	119.9	126.4	135.1
IL112 × IL101	122.6	119.0	140.9	IL103 × HKI-193-1	156.5	143.9	131.1
IL112 × IL103	120.4	130.5	119.5	IL103 × IL105	125.0	115.4	120.7
IL112 × HKI-193-1	98.8	137.3	138.3	IL103 × CM140	160.5	176.7	169.3
IL112 × IL105	121.6	117.5	122.1	IL103 × IL111	135.0	122.1	141.2
IL112 × CM140	172.3	153.8	151.7	IL103 × IL104	143.6	141.2	159.3

IL112 × IL111	142.3	149.8	130.5	IL103 × IL109	154.3	144.8	158.9
IL112 × IL104	139.4	137.1	146.7	HKI-193-1 × IL105	143.0	133.4	132.4
IL112 × IL109	115.3	124.1	137.3	HKI-193-1 × CM140	130.0	178.4	155.4
IL113 × IL101	104.7	122.0	121.8	HKI-193-1 × IL111	116.9	113.3	121.8
IL113 × IL103	110.9	96.2	118.2	HKI-193-1 × IL104	138.5	147.6	146.6
IL113 × HKI-193-1	115.0	125.8	108.5	HKI-193-1 × IL109	94.2	138.6	113.4
IL113 × IL105	136.7	129.4	133.1	IL105 × CM140	123.6	109.7	111.1
IL113 × CM140	141.6	128.7	126.8	IL105 × IL111	120.8	119.9	137.3
IL113 × IL111	140.4	146.6	150.0	IL105 × IL104	149.1	130.8	158.8
IL113 × IL104	133.0	119.6	97.5	IL105 × IL109	106.4	99.5	117.9
IL113 × IL109	130.7	137.3	136.4	CM140 × IL111	152.0	155.7	159.7
IL101 × IL103	123.5	112.2	120.9	CM140 × IL104	140.5	161.9	176.6
IL101 × HKI-193-1	112.8	116.1	123.6	CM140 × IL109	143.7	151.4	153.8
IL101 × IL105	129.1	107.4	122.8	IL111 × IL104	148.8	155.3	141.8
IL101 × CM140	148.3	144.7	156.6	IL111 × IL109	141.7	136.8	141.2
IL101 × IL111	168.4	141.8	149.7	IL104 × IL109	158.2	154.6	157.9
IL101 × IL104	144.2	141.5	135.9	**HIM 129 (Check)**	122.5	131.9	117.3

Genotype	**R I**	**R II**	**R III**	**Genotype**	**R I**	**R II**	**R III**
IL112	104.7	115.5	116.4	IL105	100.3	119.4	111.3
IL113	87.9	110.7	102.3	CM140	121.5	129.8	114.4
IL101	102.4	102.7	100.9	IL111	126.8	106.2	122.3
IL103	125.3	121.7	126.6	IL104	120.6	131.9	136.4
HKI-193-1	115.6	130.5	115.9	IL109	115.2	119.4	118.5

ANOVA Table

Source	**Degree of freedom (df)**	**Sum of squares (S.S.)**	**Mean sum of squares (M.S.)**	**Calculated F**
Replication	2	385.88	192.94	1.98
Treatment	54	44469.98	823.52	8.46
Error	108	10517.35	97.38	-
Total	164	55373.21	-	-
S.Em. = 5.70	**C.D. (0.05) = 15.97**	**C.V. % = 7.53**	**General Mean = 131.13**	
Table 't' Value @ 5% = 1.98 and @ 1% = 2.62 for error degree of freedom (108).				

EXERCISE 6: EMASCULATION AND HYBRIDIZATION TECHNIQUES IN SELF AND CROSS POLLINATED CROP Date:

- Natural variability present in self-pollinated populations is exhausted quickly when they are subjected to selection.
- For further improvement, therefore, new genetic variability has to be created, which is easily and most commonly achieved by crossing two different purelines.
- The mating or crossing of two plants or lines of dissimilar genotype are known as **hybridization**.
- In plants, crossing is done by placing pollen grains from one genotype, called the **male parent**, onto the stigma of flowers of the other genotype, referred to as the **female parent**.
- It is essential to prevent self-pollination as well as chance cross-pollination in the flowers of the female parent. At the same time, it must be ensured that the pollen from desired male parent reaches the stigma of flowers of the female parent for successful fertilization.
- The seeds as well as the, progeny resulting from hybridization are known as **hybrid.**
- The progeny of F_1, obtained by selfing or intermating of F_1 plants, and the subsequent generations are termed as segregating: generations. The term cross is often used to denote the products of hybridization, i.e., the F_1 as well as the segregating generations.

OBJECTIVES OF HYBRIDIZATION

- To create genetic variation
- To combine desirable characters into single plant (Combination breeding)
- To study the pattern of inheritance of the character
- To exploit and utilize hybrid vigour
- To produce transgressive segregants (Transgressive breeding)
- To produce hybrids in various crops for commercial cultivation by farmers
- To assess general combining ability of the parents and specific combining ability of crosses

TYPES OF HYBRIDIZATION

Based on the taxonomic relationships of the parents involved, hybridization may be classified into two broad groups:

1) Inter varietal Hybridization

- The parents involved in inter varietal hybridization belong to the same species; they may be two strains, varieties or races of the same species. It is also known as intraspecific hybridization.
- Most commonly used for crop improvement

- Examples
  - Wheat : GW 496 × GW 322
  - Rice : IR 64 × IR 8

2) Distant hybridization

- It involves individuals belongs to same genus or different genus.
- Hybridization involving individuals belongs to distinct species of the same genus, it is called interspecific or intrageneric hybridization.
  - Examples : Wheat – *Triticum aestivum* × *Triticum durum*
- Hybridization involving individuals belongs to different genus, it is called intergeneric hybridization.
  - Examples : Triticale – *Triticum aestivum* × *Secale cereal*
  - First man made cereal prepared by Rimpau (1890).
- These both interspecific and intergeneric hybridization are known as distant or wide hybridization.
- In general, distant hybridization is much more difficult than intervarietal hybridization; the difficulty increases with the taxonomic distance between the parents involved.
- Usually, the aim of such crosses is to transfer few simply inherited characters like biotic and abiotic stress tolerance genes from wild to cultivated species.

3) Introgressive hybridization

- It is one kind of inter-specific hybridization. In this type of hybridization, hybrids may have repeatedly backcrossed to one of the parental species, so the most of the nuclear genes of the parental species would be recovered along with few genes from the other parental species.
  - Example: Modern cultivated maize is developed by crossing Primitive maize with *Tripsacum* (wild weedy species).

PROCEDURE OF HYBRIDIZATION

1. Choice of Parents

- The parents chosen for a hybridization must together possess all the traits, and in sufficient intensity, which the breeder wishes to improve in the new variety.
- At least one parent must be well adapted in the region for which the variety is to be developed. The other parent(s) should possess the traits, which one wishes to improve in this well-adapted variety.
- The parents should be genetically diverse if the breeder aims to obtain transgressive segregants.
- The general combining ability of the parents should also be considered, if the estimates for the same are available. Otherwise, the level of phenotypic expression

should be used as a guide since usually per se performance for a trait is positively associated with the general combining ability for the trait.

2. Evaluation of Parents

- ❖ It is desirable to evaluate the performance of chosen parents in the area for which breeding is to be done.
- ❖ Evaluation provides the following information about the parents: (1) performance in terms of yield and yield traits, (2) reaction to the prevalent diseases and insect pests, (3) presence of mechanical mixtures and (4) existence of heterozygosity.

3. Emasculation

- ❖ Removal of stamens/anthers or inactivation of pollen grains, before the pollen grains become mature without any harm to the gynoecium, is called emasculation.
- ❖ Emasculation is done in the flowers of the parent to be used as female in a hybridization program. It is usually done one day before anthesis of the concerned flowers. The sole aim of emasculation is to prevent self-pollination in the flowers of the female parent.
- ❖ The efficiency of emasculation can be tested by bagging the emasculated flowers without pollination. The amount of seed set in such flowers will indicate the extent of self-pollination, which occurred during emasculation.
- ❖ In crop improvement programmes, however, a small amount of self-pollination may be permissible, but it should be kept to the minimum.
- ❖ An efficient emasculation technique should prevent self-pollination and produce a high percentage of seed set on cross-pollination. The different techniques of emasculation are as follows:

a) Hand Emasculation:

- ❖ In species with relatively large flowers, stamens or anthers are removed with the help of forceps. Emasculation is usually done one day before the anthesis of flowers during evening time.
- ❖ Generally, younger buds and older flowers, even pods close to the flower bud selected for emasculation are removed to avoid confusion.
- ❖ The selected bud is then opened with the help of forceps, and all the anthers are carefully removed.
- ❖ Care must be taken to remove all the anthers intact (i.e., without rupturing them) from flower. Further, no damage should be caused to the female reproductive organ, i.e., stigma and ovary.

b) **Suction Method:**

- ❖ This method is useful in species with small flowers, where hand emasculation is tedious. Emasculation is done in the morning, on the day of anthesis, just before or

immediately after the flowers open. If needed, petals may be removed with the help of forceps.

- A thin rubber or glass tube is attached to the suction hose of a suitable suction pump /aspirator. The tube is passed over the flowers to suck the anthers and also the pollen grains that may be present on the stigmas. A considerable amount (up to 15%) of self-pollination may occur with this method.

c) Hot Water Method:

- Pollen grains are more sensitive than the female reproductive organs to both genetic and environmental factors. Therefore, treatment with hot water of a suitable temperature inactivates pollen grains without reducing female fertility.
- In case of jowar (sorghum), treatment with water at 42-44°C is optimum. Hot water is generally carried in a thermos in which the whole panicle/spike (ear/head) is immersed. It is generally highly effective, but regulating the water temperature may present problems.

d) Cold Treatment:

- Exposure of wheat plants to 0-2°C for 15-24 hr inactivates pollen grains without damaging gynoecium.
- In case of rice, treatment with cold water (0-6°C) kills the pollen grains. Cold treatment is generally less effective than hot water treatment; it also shows a higher frequency of self-pollination.

e) Chemical Treatment / Gametocides:

- Certain chemicals, which are sprayed at the time of flowering induces male sterility plant.
- Gametocide: the chemicals which are used to kill the pollen grains are known as gametocides.

Gametocide / CHA	**Chemical formulation**	**Crops**
Arsenicals	Zinc methyl arsenate, Sodium methyl arsenate	Rice
Dalapon	Sodium 2,2- dichloro propionate	Cotton, Pearl millet, Wheat, Onion
Ethepon	2-chloroethyl phosphonic acid	Wheat, Barley, Rice, Oats
FW 450 (Mendok)	Sodium α, β -dichloto isobityrate	Capsicum, Cotton, Pearl millet, Sesame, Sunflower
OMT	L-O-Methyl threonine	Cotton
MH	1,2-dichloropyridazine-3,6-dione	Maize, Wheat, Cotton
GA	Gibberellic acid	Maize, Barley, Wheat, Rice,

- ❖ In clovers, a 10 second treatment with 57% alcohol was quite effective; the frequency of self-pollination was less than 1%.
- ❖ Care must be taken that the chemical must not affect the function of the female reproductive organ. The duration of treatment should be strictly controlled, since even slightly longer duration would damage gynoecium and reduce seed set.

f) **Genetic Emasculation:**

- ❖ Prevention of self-pollination by genetic means is called genetic emasculation.
- ❖ This is achieved by using genetic (including transgenic) or cytoplasmic-genetic male sterility, self-incompatibility or pistillate condition. **This technique is of great importance in hybrid seed production.**

4. Bagging:

- ❖ The emasculated flowers or the whole inflorescences are enclosed in suitable bags; this is called bagging.
- ❖ Bagging is done to prevent chance cross-pollination. Bags may be made of paper, butter paper, glassine or fine cloth. The bags are tied to the base of inflorescence or to the stalk of flower with the help of thread, wire or pins (of a suitable design).
- ❖ The bags are usually removed few days after pollination to prevent fungus growth. In cross-pollinated crops like maize, the male flowers are also bagged to ensure the purity of pollen used for pollination.

5. Tagging:

- ❖ The emasculated flowers are tagged just after bagging. In most crops circular tags of about 3 cm diameter or rectangular tags of 3 × 2 cm are used.
- ❖ In crops like Maize, Bajra etc., larger tags of 6 × 3 cm are used. The tags are attached to the base of inflorescence or the stalk of flowers with the help of a thread.
- ❖ The following information is recorded on the tags with a carbon pencil:
    - o Date of emasculation,
    - o Date of pollination, and
    - o Names of female and male parents [in that order, i.e., A (female parent) x B (male parent)].

6. Pollination:

- ❖ Transfer of mature and active pollen from the flowers of male parent on to the receptive stigmas of emasculated flowers is called **pollination.**
- ❖ It is essential for seed set in the emasculated flowers. Pollination is usually done in the morning at the time of anthesis, next day following emasculation.
- ❖ In Bajra, etc., pollens are collected in a bag and dusted onto the stigmas of female flowers. In wheat, barley, etc., one mature anther about to burst is inserted in each floret. Alternatively, the spike of male parent may be shaken over the emasculated spike; this is done when lemma and palea are clipped off during emasculation. In

species like Maize, the male inflorescence may be detached and enclosed in the bag covering the female inflorescence.

- ❖ Mature anthers are collected, pollen liberated and applied to the stigmas using camel hairbrush, tooth pick, pieces of paper, etc.

7. Harvesting and Storing the F_1 Seed:

- ❖ The crossed heads/spikes/pods are harvested, threshed, dried and stored properly.
- ❖ Care should be taken to protect them from storage pests and from moisture, especially during the monsoon.
- ❖ Every precaution should be taken to prevent contamination from other seed. Seeds from each cross should be handled separately, and the original tags should be kept with them.

Types of crosses

❖ **Single Cross**:

- It is a cross between to genetically dissimilar homozygous plants
  e.g. $A \times B = F_1$.
- Concept was proposed by G. H. Shull (1909) in Maize.
- Number of possible single crosses without reciprocals = **[n(n-1)] / 2**
- Number of possible single crosses with reciprocals = **[n(n-1)]**
  Where, n = Number of parents

❖ **Double cross:**

- It is a cross between two different single crosses.
  e.g. **(**$A \times B) \times (C \times D) = F_1$ (Double cross)
- Concept was proposed by D. F. Jones (1909) in Maize.
- Number of possible double crosses = **[n(n-1)(n-2) (n-3)] / 8**
  Where, n = Number of parents

❖ **Three-way cross:**

- When F_1 from a single cross is mated to a third parent, it is called **three-way cross**.
  e.g. **(**$A \times B) \times C = F_1$ (Three way cross)

❖ **Multiple cross or Complex Cross**:

- When more than four parents are crossed to produce the F_1 hybrid.
  e.g. **(**$A \times B) \times$ **(**$C \times D) \times$ **(**$E \times F) \times (G \times H) = F_1$
- It aims at bringing together genes from several parents into a single hybrid and also create large amount of genetic variability.

❖ **Back Cross**:

- A cross between F_1 and any one of its parents.
  e.g. **(**$A \times B) = F_1$ $\quad$ **$F_1 \times A = BC_1$** $\quad$ **or** $\quad$ **$F_1 \times B = BC_2$**
- It is used to transfer specific deficient trait from donor parent to recipient parent.
- To develop isogenic lines for multiline development.

- To develop Near Isogenic lines (NILs) for mapping population.

❖ **Test Cross**:

- A cross between F_1 and its homozygous recessive parents.
  e.g. **(**AA × aa) = F_1 **F_1 × aa = Test cross progeny**
- It is used for linkage study.

❖ **Top Cross**:

- A cross made between an inbred line and open pollinated variety.
- It was proposed by Davis (1929).
- It is used to study the general combining ability of inbred lines.

❖ **Double top Cross**:

- A cross made between single cross hybrid (F_1) and open pollinated variety.

❖ **Poly Cross**:

- The open pollination group of genotypes in isolation from other compatible genotypes in such a way to promote random mating. In this, all the genotypes have equal chance of pollination.
- It was proposed by Tysdal, Kiessel bach and Wastover (1942).

Exercise:

1. Define : Hybridization, Single cross, Interspecific hybridization, Genetic emasculation, Gametocides, Double cross, Top cross
2. Objectives of hybridization
3. Explain procedure of hybridization in crops.
4. Explain different techniques of emasculation
5. Calculate the number of single crosses, three way crosses and double crosses generated using ten parents.

EXERCISE 7: CONCEPTS OF POPULATION GENETICS AND HARDY – WEIGNBERG LAW Date:

- Cross-pollinated crops are highly heterozygous due to the free intermating among their plants. They are often referred to as random mating populations because each individual of the population has equal opportunity of mating with any other individual of that population. **Such a population is also known as Mendelian population or panmictic population.**
- A Mendelian population may be thought of having a gene pool consisting of all the gametes produced by the population. Thus, **gene pool** may be defined as the sum total of all the genes present in a population.

Hardy - Weinberg Law

This law was independently developed by Hardy (1908) in England and Weinberg (1909) in Germany. This law states that **"In the large random mating population, gene and genotypic frequencies remain constant generation after generation in the absence of selection, mutation, migration and random genetic drift".**

FACTORS AFFECTTING THE EQUILIBRIUM IN THE POPULATION :

1. **Selection:** Differential reproduction rates of various genotypes is known as selection.
2. **Migration:** The movement of individuals into a population from a differential population, and participation in the reproduction of this population. It may introduce new alleles into the population or may change frequencies of existing alleles.
3. **Mutation:** A sudden and heritable change in any character of an organism and is generally due to a structural change in the concerned gene. It is the ultimate source of all the variation present in biological materials. It may produce a new allele that was not present in the population or may change existing allelic frequencies. However, since mutation rate is generally very low, i.e., approximately 10^{-6}, the effects of mutation on gene frequency would be detectable only after a large number of generations. Therefore, in breeding populations such effects may be ignored.
4. **Random genetic drift:** It is a random change in gene and genotypic frequency due to sampling error. In a smaller population if natural selection operates at random it will lead to sampling error. This sampling error is greater in smaller population than in a large one. Because of sampling the frequency of one of the alleles becomes zero and that of the other alleles become one. The allele having the value one is said to be fixed because there is no further change in its frequency and thus it becomes homozygous. Thus, if the population is small genetic drift will occur. To overcome this, one has to use larger population, which may not be possible because of limitations in space, labour and finance.

Apart from in smaller populations, a certain amount of inbreeding is bound to occur and this will lead to homozygosity. ***Mating between individuals sharing a common parent in their ancestry is known as inbreeding***. Inbreeding reduces the proportion of heterozygotes or heterozygosity and increases the frequency of homozygotes or homozygosity. Thus, in small populations, even with strict random mating or even with strict cross-pollination the frequency of homozygotes increases, while that of heterozygotes decreases due to inbreeding.

- In a species for a single gene with two alleles, A and a in a random mating population, there would be three possible genotypes AA, Aa and aa.
- If the frequency of **allele A** is denoted as "**p**" and of **allele a** as "**q**" in the population.
- The frequencies of these three genotypes would be AA = p^2, Aa = 2pq and aa = q^2
  If, the sum of gene frequencies, i.e. p and q is one i.e. **p + q =1** then sum of its genotypic frequencies would be $\mathbf{p^2 + 2pq + q^2 = 1}$.

When the gene and genotypic frequencies remain constant from one generation to next, such a population would be called in equilibrium. This equilibrium is known as Hardy-Weinberg equilibrium.

Whether the population is at equilibrium or not, it can be tested by chi-square test.

- Suppose the population has N individuals of which total number of individuals similar to **AA** denoted as **D**, **Aa** denoted as **H** and **aa** denoted as **R.**
- So, N = D + H + R. The total number of alleles at this locus in the population would be 2N since each individual has two alleles at a single locus.
- The total number of A alleles would be 2D+H because AA Individuals have two A alleles each, while each Aa individual has only one A allele.
- Therefore, the gene frequency of allele A is **p = (2D+H)/2N or (D+1/2H)/N**
- Similarly, the gene frequency of allele a is **q = (2R+H)/2N or (R+1/2H)/N**
- **Whereas, genotypic frequency for AA is p^2 = D/N and genotypic frequency for aa is q^2 = R/N.**
  - ❖ **Gene frequency** is the proportion of an allele, A or a, in a random mating population or the proportion of gametes carrying an allele, A or a.
  - ❖ **Genotype frequency** or **zygotic frequency** is the relative proportion of a genotype, AA, Aa or aa, in the population.

Characteristics of random mating population

1. Each genotype in the population is different in some gegree from others.
2. Each genotype is highly heterozygous.
3. Each genotype is largely out crossing or random breeding or cross pollinating.
4. Each genotype gives a variable progenies.

   Hence, gene and genotypic frequencies are important in cross pollinated crops rather than genetic inheritance in self-pollinated crops.

Calculation of gene and genotypic frequency

Example I:

In a sample population, total numbers of individuals are 100 which includes AA = 30, Aa = 10 and aa = 60

So, D = 30, H = 10, R = 60 and N = 100

Gene frequency of A Individual allele

$p = \mathbf{[D + (\frac{1}{2}H / N]}$

$= [30 + \frac{1}{2}(10) / 100]$

$= 35 / 100 = 0.35$

Similarly for 'a' individual allele

$q = \mathbf{[R + (\frac{1}{2}H / N]}$

$q = [60 + \frac{1}{2}(10) / 100]$

$= 0.65$

∴ Here allele frequency, $p + q = 1$ ∴ $0.35 + 0.65 = 1$

Exercise:

1. What is the frequency of heterozygotes. (Aa) in mendelian population. If the frequency of recessive phenotype (aa) is 0.16?
2. Answer the following based on given data

a)

Population	**No. of Individuals**		
	AA	**Aa**	**aa**
1	0.64	0.32	0.04
2	0.46	0.24	0.36

Which of the above population is in Hardy-Weinberg equilibrium?

b) What are the expected equilibrium frequencies for those above population are not in equilibrium?

c) How long will it take for this equilibrium value to be reached at random population?

3. Answer the following questions based on the date given to you.

Population	**No. of Individuals**		
	AA	**Aa**	**aa**
1	600	459	207
2	250	400	750

a) Calculate the genotype frequencies for above genotypes.

b) Calculate the gene frequencies for above genes/alleles.

c) What is the expected number of each genotype if the above population is in Hardy Weinberg Equilibrium?

4. The following numbers of the human M-N blood groups were recorded in a sample of Africans White.

MM	MN	NN
1787	3039	1303

a) What are the genotype frequencies observed in this sample?
b) What are the gene frequencies observed in this sample?
c) With the gene frequencies observed, What are the genotype frequencies expected from the Hardy - Weinberg law ?
d) How well do the observed frequencies agree with the expected ?

5. Explain the factors disturbing the H-W equilibrium in the population.
6. Explain Hardy - Weinberg law with example.

EXERCISE 8: METHODS OF CALCULATING MEAN, RANGE, VARIANCE, STANDARD DEVIATION Date:

- When different plants/lines of a species show different magnitudes of a character, it is called **variation.**
- Variation is essential for any improvement in a species. Therefore, the first step in any breeding programme is to create variation if it is not already present in the population to be improved.
- Variation can be created by hybridization, mutation, polyploidy, domestication. plant introduction, somaclonal variation, genetic engineering, etc.

1. Mean of the distribution (First degree statistics)

i. **Arithmetic mean:** It is defined as sum of all observation divided by the total number of individual added.

$$\bar{X} = \frac{\sum X_i}{N}$$

Where, $\bar{X}$ = Mean , $\sum X_i$= sum of the individual observations

N= total number of observations

ii. **Mode:** Most frequent value in the population.

iii. **Median:** The middle value in an array of observation.

Frequency distribution = $\frac{\sum fx}{N}$

Where, f = Class frequency

x = Class value

2. Measures of dispersion (Second degree statistics)

i. **Range :** The difference between the values of the highest and the lowest observations of a sample is called **range.** It is generally depicted by listing the values of the highest and the lowest observations.

ii. **Mean deviation or Average deviation :** Mean deviation is the average of deviation of individual observation from the mean.

Mean deviation = $\frac{\sum(x-\bar{x})}{N}$

Where, x is the individual observation

$\bar{x}$ = is the mean

Mean deviation by frequency = $\frac{\sum fd}{N}$

Where, f= Class frequency

d= deviation of class value from the mean

iii. **Standard deviation :** It is the square root of variance and is designated as SD in case of sample, or as σ in case of population.

Standard Deviation (S.D.) = $\sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{N}}{N-1}}$

iv. **Variance :** average of square deviation of all the individual observation from the mean.

Variance $= \frac{\sum x^2 - \frac{(\sum x)^2}{N}}{N-1}$

N- 1 = degree of freedom

v. **Standard error :** It is the measure of the mean difference between sample estimate of mean ($\bar{X}$) and the population parameter (μ) *i.e.* it is the measure of uncontrolled deviation present in a sample.

Standard error = $\frac{Standard\ deviation}{\sqrt{N}}$

It is necessary to describe any sample in terms of its mean ± standard error.

vi. **Coefficient of variation (C.V. %) :** It is a percentage ratio of standard deviation to the arithmetic mean of a given series. It is without unit or unit less.

$$C.V.\ \% = \frac{Standard\ deviation}{Mean} \times 100$$

vii. **Covariance:** Any pair of related characters may yield a covariance

Example:

The plant height of 30 rice genotypes is given below. Workout the biometrical quantities *viz.*, mean, variance, standard deviation, standard error and coefficient of variation.

Genotype	Plant height (cm)	Genotype	Plant height (cm)	Genotype	Plant height (cm)
1	115.9	11	102.5	21	98.6
2	120.8	12	111.5	22	107.3
3	101.2	13	125.2	23	115.6
4	106.7	14	104.9	24	128.3
5	113.3	15	87.7	25	93.7
6	110.7	16	110.6	26	108.3
7	96.9	17	102.7	27	112.6
8	117.9	18	98.3	28	100.5
9	128.1	19	120.6	29	115.4
10	97.7	20	89.7	30	96.7

Answer:

(a) Arithmetic mean $= \frac{\sum x}{N}$

$= \frac{115.9+120.8+\cdots\ldots+96.7}{30}$

$= \frac{3239.90}{30}$

$= 108.00$

Frequency Distribution

Range	Class value (x)	Frequency (f)	fx	d	fd	fd^2
85-90	87.5	2	175.0	-20.83	-41.66	867.78
90-95	92.5	1	92.5	-15.83	-15.83	250.59
95-100	97.5	5	487.5	-10.83	-54.15	586.44
100-105	102.5	5	512.5	-5.83	-29.15	169.94
105-110	107.5	3	322.5	-0.83	-2.49	2.07
110-115	112.5	5	562.5	4.17	20.85	86.94
115-120	117.5	4	470.0	9.17	36.68	336.36
120-125	122.5	2	245.0	14.17	28.34	401.58
125-130	127.5	3	382.5	19.17	57.51	1102.47
Total		**30**	**3250.0**		**0.10**	**3804.17**

Mean = $\frac{\sum fx}{\sum f}$

$= \frac{(2\ x\ 87.5)+(1+92.5).....+(3+127.5)}{30}$

$= \frac{3250.0}{30}$

= 108.33

(b) Range = 87.7 to 128.3

(c) Variance $= \sum x^2 - \frac{(\frac{\sum x}{N})^2}{N-1}$

$= \frac{115.9^2 + 120.8^2 .....+ 96.7^2 - \frac{(3239.9)^2}{30}}{29}$

$= \frac{353345.05-349898.40}{29} = 118.85$

Variance by frequency distribution = $\frac{\sum fd^2}{\sum f} = \frac{3804.17}{30} = 126.81$

(d) Standard deviation = $\sqrt{\text{Variance}}$ = $\sqrt{118.85}$ = 10.90

(e) Standard error $= \frac{\text{Standard deviation}}{\sqrt{N}}$

$= \frac{10.90}{5.48}$

= 1.99

(f) Coefficient of variation (C.V.%) = $\frac{\text{Standard devaition}}{\text{Mean}}$ x 100

$= \frac{10.90}{108}$ x 100 = 10.09 %

EXERCISE 9: COMPONENT OF GENETIC VARIATION – HERITABILITY AND GENETIC ADVANCE Date:

Variability:

It means a difference among the individuals belonging to a single species or different species within population.

The variability may be due to genotypes, environment pr due to the interaction of genotype with environments. There are two basic types of variability

1. **Genotypic variability:** It is the component of variability, which is due to genetic differences among the individuals within a population. It is the main concern of the plant breeder.
2. **Phenotypic variability:** It is the observable variation present in a character of a population. It includes both genotypic and environmental components of variability and as a result, its magnitude differs under different environmental conditions.

   Total variability can be partitioned into following components as under:

   A. Genotypic variability:
      a. Additive component
      b. Dominance component
      c. Epistatic component
   B. Environmental variability

Importance of genetic variability in Plant Breeding

- The progress of any plant breeding programme for crop improvement depends on the extent of the genotypic variability present in the base population.
- The assessment of the genetic variability present in the base population is the pre-requisite before the adoption of the selection pressure in the variable population and efficiency of the selection depends upon the identification of genetic variability from the phenotypic expression of the character.
- The study of the genetic variability is necessary to determine the relative importance of various plant characters with respect to yield in terms of genetic variability.
- The parameter like range of variability, co-efficient of variability, heritability and expected genetic advance are computed for the assessment of the genetic variability and gene action in expressing the genotypes which reflects into effective selection in crop improvement programme.
- Genetic variability for important agronomic traits in almost all the crop is mainly due to the additive genetic variance. The non-additive variance also exists for many important traits in nearly all the crop but it is generally smaller in magnitude than additive component.
- The variation required for improvement in any crop can be generated through plant introduction, hybridization, mutation, polyploidy as well as somatic variation.

- Estimation of phenotypic and genotypic variances helps in the computation of heritability and genetic advance.

Estimation of genetic variability, Heritability and genetic advance:

The genetic variability present in breeding populations can be assessed by three ways:

a) By using simple measure of variability
b) By estimating the various components of variance
c) By studying the genetic diversity within a population

1. By using simple measure of variability

When the experimental material is not replicated, the phenotypic (total) variability present in breeding material can be assessed by using following simple measures of variability. e.g. range, arithmetic mean, standard deviation, coefficient of variation, standard error

2. By estimating the various components of variance

The magnitude of phenotypic variation present in a breeding population may be partitioned into several components of variation using appropriate experimental design in replicated trials. In randomized block design (RBD), the structure of analysis of variance (ANOVA) is as under.

Source	**d.f.**	**S.S.**	**M.S.**	**Cal. F**	**Expected M.S.**
Replication	r-1	Rss	M_r = Rss/ r-1	M_r / M_e	$\sigma_e^2 + g\sigma_r^2$
Genotype	g-1	Gss	M_g = Gss/ g-1	M_g / M_e	$\sigma_e^2 + r\sigma_g^2$
Error	(r-1) (g-1)	Ess	M_e = Ess/ (r-1) (g-1)	--	σ_e^2
Total	rg-1	Tss	--	--	--

A. S.Em. = $\sqrt{\frac{Error\ M.S.}{r}}$

B. C.D. = S.Em. x $\sqrt{2}$ x $t_{0.05}$ at error d.f

C. Coefficient of variation (CV %) = $\frac{\sqrt{Error\ M.S.}}{\bar{X}} \times 100$

D. Error variance (Environment variance) (σ_e^2)**:** It is the non-heritable variation which is due to the environment which varies depending upon the environments. $\sigma^2_e = M_e$

E. Genotypic variance (σ_g^2) : It is only measures the amount of genotypic variability present in a character of a population.

$\sigma_g^2 = \frac{(M_g - M_e)}{r}$

F. Phenotypic variance (Total variance) (σ_p^2) **:** It is the total variation which is observable and is the sum total of genotypic and environmental variances.

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

G. Genotypic coefficient of variation (GCV %) : It is measure the range of genetic variability present in a character of a population and also provides a measure to compare the genetic variability among various plant characters within and between different populations. it is calculated as

$$\frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$$

H. Phenotypic coefficient of variation (PCV %) :

$$\frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100$$

GCV % and PCV % are classified as suggested by Sivasubramaniam and Madhavamenon in 1973 as

Low	Less than 10 %
Moderate	10 – 20 %
High	More than 20 %

If the difference between GCV and PCV is less, it indicates that environment has little influence on the characters and if the difference is more it states that environment plays a substantial role in the expression of that character.

I. Heritability is the proportion of the total variability that is due to genetic causes (or) it is the ratio of genotypic variance to total variance.

- It is a good index of transmission of characters from parents to their offspring. Depending upon the components of variance used as numerator in the calculation of the heritability, it is of two types:

1) <u>Broad sense heritability</u> is the ratio of genotypic variance to the total phenotypic variance and is calculated as

Broad sense heritability (h_b^2) (%) = $\frac{V_g}{V_p}$ x 100 or $\frac{\sigma^2_g}{\sigma^2_p} \times 100$

Where, $V_g = \sigma^2_g$= Genotypic variance and $V_p = \sigma^2_p$= Phenotypic variance

The features of broad sense heritability:

1. It can be estimated from both parental as well as segregating populations
2. It is estimated from total genetic variance
3. It is more useful in animal breeding than in plant breeding
4. It is useful in the selection of elite types from homozygous material

2) <u>Narrow sense heritability</u> is the ratio of additive (or) fixable genetic variance to the total phenotypic variance and is calculated as

Narrow sense heritability (h_n^2) (%) = $\frac{V_a}{V_p}$ x 100 or $\frac{\sigma^2_a}{\sigma^2_p} \times 100$

Where, $V_a = \sigma^2_a$= Additive genetic variance and $V_p = \sigma^2_p$= Phenotypic variance

The features of narrow sense heritability:

1. For estimation of narrow sense heritability, crosses have to be made in definite fashion
2. It is estimated from additive genetic variance.
3. It is useful in both, plant breeding as well as animal breeding.
4. It is useful in the selection of elite types from segregating populations.

Difference between broad sense and narrow sense heritability are given as:

	Broad sense heritability	**Narrow sense heritability**
1.	Estimated from total genetic variance	Estimated from additive genetic variance
2.	Can be estimated from both parental and segregating material	Requires crossing in definite fashion
3.	More useful in animal breeding	Useful in both plant and animal breeding
4.	Useful in selection of elite types from homozygous lines	Useful in selection of elite types from segregating material

Heritability plays an important role in selection process in the plant breeding especially in the selection of elite genotypes from segregating populations. Johnson *et al.,* (1955) categorized heritability as

Low	Less than 30 %
Moderate	30 – 60 %
High	More than 60 %

J. Genetic advance (GA):

It is a measure of genetic gain under selection. It refers to the improvement in the mean genotypic value of the selected lines (or) families over the base or over population.

The expected genetic gain (or) advance under selection may be estimated as suggested by Johnson as

GA= k × σ_p × h_b^2

Where, h_{bs}^2 = Broad sense heritability

k = Selection differential at 5% intensity of selection, k = 2.06

Genetic advance is also calculated by another formula

Genetic advance (GA) = k x $\sqrt{\sigma^2_p}$ x h_b^2

Where, VP = phenotypic variance

K. GA as a percentage of mean : $\frac{GA}{\bar{X}} \times 100$

The success of genetic advance under selection depends

- The magnitude of genetic variability present in base population
- Heritability of a character under selection.
- Intensity of selection

The range of genetic advance is classified by Johnson *et al.*, 1955 as

Low	Less than 10 %
Moderate	10 – 20 %
High	More than 20 %

Heritability and genetic advance are important selection parameters and heritability estimate along with genetic advance are interrupted as follows:

1) High heritability accompanied with high genetic advance indicates heritability is due to additive (or) fixable variation and selection may be effective.
2) High heritability accompanied with low genetic advance indicates non additive gene action and selection for such characters may not be rewarding.
3) Low heritability accompanied with high genetic advance reveals that characters are governed by fixable gene effects and low heritability is due to high environmental influence and selection may be effective.
4) Low heritability accompanied with low genetic advance indicates that character is highly influenced by environment and selection is ineffective.

3. By studying the genetic diversity within a population

There are two important biometrical techniques used for this purpose are as under

a) Metroglyph analysis and index score method
b) D^2 statistics by Mahalanobis, 1936

Exercise:

1. Estimate Variability parameters viz., Error variance, Genotypic variance, Phenotypic variance, GCV, PCV, Heritability (broad sense), expected genetic advance and GA expressed as % of mean from the following data and write conclusion based on the result.

ANOVA				
Source of Variation	**df**	**S. S.**	**M. S.**	**CAL. F**
Replication	2	3.22	1.6	58.18
Genotype	24	2708.59	112.86	
Error	48	93.28	1.94	
Total	74	2805.09	**General mean** = 50.76	

2. Define variability, heritability, PCV, Genetic advance.
3. Differentiate : Broad sense and Narrow sense heritability

EXERCISE 10: DESIGNS USED IN PLANT BREEDING EXPERIMENTS

Date:

Terminologies:

- **Treatment:** The objects of comparison which an experimenter has to try out in the field for assessing their values are known as **Treatment**. e.g., varieties, manures, cultivation practices, methods of seed treatments, insecticides, etc.,
- **Experimental material:** The material on which the experiment is performed is called experimental material. *e.g.* a set of varieties, a set of various sources of nitrogenous fertilizer, a set of animals, etc.
- **Experimental Units:** The experimental material is divided into a number of ultimate smaller units to which the treatments are applied is known as experimental Units. The experimental unit may be a plot of land, a patient in hospital, a cow, a group of pigs in pen, a batch of seeds, etc.
- **Uniformity trial:** A trial consists of growing in a field a particular crop with a uniform treatment, dividing the field into small units, harvesting and recording the observations from each of these units separately, is called uniformity trial. It is used to prepare "Fertility Contour Map" representing appreciable variation in fertility and it does not follow any systematic pattern and distributed over the field in an erratic fashion.
- **Experimental error:** Variation arises due to uncontrolled factors.

Basic principles of field experimentation:

There are three basic principles of field experimentation.

1) **Replication :**
   - The repetition of the treatments under investigation is known as Replication. The advantages of replications are as under.
   - In order to obtain a greater precision in the field experiments, the most effective method is to increases the number of replications. This will reduce the error. Increase in replications increases the degree of freedom of error, which decreases the value of t due to decrease in confidence interval. The shortening of confidence interval is good proof of increased precision.
   - The error of experiment arises from the difference between the plots of the same treatment. Thus without replications estimates of error is not possible and without estimates of error comparison is not possible.
   - Another way of reducing the experimental error is to increase the plot size, but the plot size should not increases beyond the 0.1 acre because further increase in plot will increase the heterogeneity within the plot and affect the advantage of increase plot size.

2) **Randomization :**

- The allocation of different treatments to the different experimental plots by a random process is known as randomization of the treatments.
- It gives equal chance to all the treatments for being allotted to a more fertile plot or plot with similar fertility.

3) **Local Control :**

- The principle of making use of greater homogeneity in groups of experimental units (e.g. blocks of a number of plots homogeneous within themselves) for reducing the experimental error is known as **'Local Control'**. •
- As a lower experimental error helps in detecting the smaller real differences between the treatments, it is desirable that it should be reduced as far as practically possible. This is possible by making use of the principle that the adjacent areas are relatively more homogeneous than those widely separated. In the field we find that the fertility of the field may be classified in two, viz.,
  a) A major fertility variation which is usually marked by a fertility gradient.
  b) Sporadic or scattered fertility variations, which are not systematic but are distributed in patches throughout the field.
- Now, if we divide the whole field into blocks, which are homogeneous within them, we can calculate the variance in yield due to the effects of the first type of fertility variation and eliminate this variance for arriving at an estimate of the variance due to chance error only.
- Randomizing the treatments within the blocks minimizes the effects of scattered fertility variations.

Experimental design: The choice of experimental design depends on the number and nature of the treatments under study. It also depends on the object of the experiment.

The following designs are used under specific situations:

1. **Completely Randomized Design** (**CRD**)**:** Used when the experimental material is limited and homogenous e.g. pot experiments on soil, laboratory experiments, etc.
2. **Randomized Block Design (RBD):** It is used when the fertility gradient in the field is in one direction. It may be adopted up to 20 treatments without any appreciable loss of efficiency.
3. **Latin Square Design (LSD):** It is used when the fertility gradient is in two directions. It may adopt for number of treatments ranging from 5 to 12 in most of the situations.
4. **Factorial Experiment:** When there are several factors with different levels to be studied simultaneously with the same precision.
5. **Split Plot Design (SPT):** It is used when the factors are such that some of them require larger plots (like irrigation, depth of ploughing, sowing dates etc.) and some require smaller plots may be studied with different precision.

6. **Incomplete Block Design (IBD):** It is used when the number of treatments is sufficiently large.

Size and Shape of plots:

- There is no particular size or shape of the plots which may be said to be the best for all circumstances.
- The plot size increases, coefficient of variation (CV) of plots decreases. Therefore, it is better to increase the plot size upto the extent of 1/ 10 acre.
- If the plot size is greater than this, the increase in soil heterogeneity within the plots affects any advantage obtained by increasing the plot size.
- However, an increase in the number of replications with reduced plot size leads to a more precise treatment comparison, when the land and other facilities are limited.
- So far as the shape of the plot is concerned it can be anything, square, rectangle or a narrow long strip.
- The dimensions of the plots are so chosen as to utilize the whole of the experimental site effective and give a correct field lay out.

Border Effect:

- The yield or any other character of the plants is affected in case of those plants which are nearer to the borders of the plots with the result that the border plant differ from the plants of the central portions of the plots with respect to the yield or other character under study.

Exercise:

1. Define : Treatment, Experimental material, Experimental Unit, Replication, Randomization, Border effect
2. Write in brief about the basic principles of experimentation.
3. Enlist the advantages of replication during experimentation.
4. Enlist the use of various experimental designs in different situations.

EXERCISE 11: ANALYSIS OF RANDOMIZED BLOCK DESIGN Date:

Lay out of RBD:

- The experimental material (field) is first divided into blocks consisting of homogeneous (uniform) experimental units.
- Each block is divided into number of plots equal to the total number of treatments.
- **Randomization** is done within each block and the treatments are applied in random manner.

Collection and analysis of data

- After the collection of data from the individual experimental units (treatments), tabulation and analysis is done. ANOVA (Analysis of Variance) table is formed.
- The significance of ANOVA table is that it indicates the sources of variation exhibited by the treatments, the magnitude of variation derived from different sources and their significance/non-significance basis.

Computation of Critical Difference (C.D.):

- Critical Difference is the difference between the treatment means, which places the treatments statistically as well as significantly apart.
- Otherwise if the difference of two treatment means is less than the C.D., it can be concluded that both the treatments are at par.

Example: Seven wheat varieties were evaluated with RBD with seven replications at governmental experimental farm. Analyse the data and write conclusions.

	Replication							
Genotype	**I**	**II**	**III**	**IV**	**V**	**VI**	**Total**	**Avg.**
GW 322	148	132	148	132	1322	124	816	136.00
GW 496	132	156	124	100	116	124	752	125.30
GW 451	132	156	116	76	132	116	728	121.30
GW 273	132	164	100	68	100	124	688	114.67
GW 278	124	124	148	44	76	100	616	102.67
GW 173	100	124	124	92	68	92	600	100.00
GW 452	116	124	160	44	100	100	636	106.00
Total	884	980	920	556	716	780	4836 **(G.T.)**	

Answer:

- General Mean (G. M.) = $\frac{4836}{42}$ = 115.14
- Correction factor (C.F.) = $\frac{(GT)^2}{N} = \frac{(4836)^2}{42}$ = 556830.86
- Total sum of square

Total SS = $\sum x^2$ – C.F. = $[(148)^2 + (132)^2 + (132)^2 + \ldots.+ (100)^2] - 556830.86$
= 34289.14

- Treatment sum of square = $\frac{\sum T^2}{r}$ – C.F. = $\frac{3378640}{6}$ - 556830.86 = 6275.81
- Replication sum of square = $\frac{\sum R^2}{t}$ – C.F. = $\frac{4018448}{7}$ - 556830.86 = 17233.14
- Error SS = Total S.S. –Treat S.S. – Rep. S.S.= 34289.14 - 6275.81 – 17233.14
= 10780.19

ANOVA Table

Source	**df**	**S.S.**	**M.S.**	**Cal. F**	**Table F**
Replication	(r-1)= 5	17233.14	3446.63	9.6	2.53
Treatment	(t-1) =6	6275.81	1045.97	2.91	2.42
Error	(t-1) (r-1) = 30	10780.19	359.34		
Total	(rt-1) =41	34289.14	836.32		

Calculated F for the treatment 2.91> Table $F_{0.05,6,30}$ value i.e. 2.42
Observed difference is significant. We conclude that treatment means are significantly differed from each other.

S.Em. = $\sqrt{\frac{MS_e}{r}}$ = $\sqrt{\frac{359.34}{6}}$ = 7.74

C. D. = table $t_{(0.05)(30)} \times \sqrt{2} \times$ S.Em.
= 2.042 × 1.41 × 7.74
= 22.348

1	2	3	4	7	5	6
136.00	125.30	121.30	114.67	106.00	102.67	100.00

Treatment 1 gives higher yield and treatment 6 gives lower yield.
Conclusions: Treatments 1, 2, 3, 4 were at par; treatments 2, 3, 4, 7 were at par; treatments 3, 4, 7, 5, 6 were at par.

C.V. % = $\frac{\sqrt{MS_e}}{G.M.} \times 100 = \frac{\sqrt{359.34}}{115.14} \times 100$ = 16.46 %

EXERCISE 12: DEVELOPMENT OF HYBRIDS & PREDICTION OF PERFORMANCE OF DOUBLE CROSS HYBRIDS

Date:

DEVELOPMENT OF HYBRID VARIETIES

Development of hybrid varieties differs from species to species. The production of hybrid varieties in cross pollinated crops consists of three main steps.

1. Development of Inbreds :

- ✓ Development of inbreds is an important step in the production of hybrids. There are two methods of developing inbred lines.
- ✓ One by selfing of heterozygous populations and another by doubling of haploids. Various populations, *viz.*, open pollinated varieties, synthetic varieties or any other heterozygous population can be used for selfing. Superior plants on the basis of vigour, disease resistance and yield are selected and selfed. Progeny of selected plants are grown separately from the selfed seed in the next season.
- ✓ Again superior plants are selected in each progeny and selfed. In this way, selfing is continued for 6-7 generations to get superior homozygous inbreds. The main purpose of selfing is to fix desirable genes in homozygous condition and eliminate genotypes with undesirable deleterious genes.
- ✓ Inbreds can also be developed from haploids by doubling the chromosome number through colchicine treatment. This is the short cut method of developing inbreds.
- ✓ The vigour which is lost during inbreeding is regained when two unrelated or diverse inbred lines are combined to develop F_1 hybrid.

2. Evaluation of Inbred lines :

- ✓ The value of an inbred is assessed from its performance in hybrid combination with other inbreds. The inbred lines are evaluated on the basis of their general combining ability (gca) and specific combining ability (sca).
- ✓ Two methods, viz. (i) Top cross method, and (ii) Single crosses are commonly used to measure the combining ability of inbreds.

(i) Top Cross Method :

- ✓ Top cross refers to a cross between an inbred line and an open pollinated variety. Several inbred lines say 100 are crossed to a common tester (open pollinated variety) to produce 100 single crosses.
- ✓ The yield performance of these crosses is evaluated in replicated trials on multiple locations. Those lines which produce high yielding single cross with tester are selected. This method was suggested by Davis in 1927.
- ✓ A large number of lines can be evaluated by this method at a time. The yield is compared from the mean yield of all the crosses.
- ✓ Inbred lines which give high yield in top crosses generally produce high yielding

single crosses. This method is used for measuring general combining ability.

- ✓ Here combining ability refers to yield performance and not to gca variances and effects. The top cross seed is produced by planting alternate rows of inbred and open pollinated variety and removing the tassel (male inflorescence) of inbred line.

(ii) Single Cross Method:

- ✓ This method is used to measure the specific combining ability of those inbreds which are selected on the basis of top cross performance.
- ✓ The selected lines are crossed in all possible combinations *viz*, n(n - 1)/2, where n is the number of inbred lines.
- ✓ With 10 inbred lines, there would be 10(10 - 1)/2 = 45 single crosses excluding reciprocals.
- ✓ These single crosses are evaluated in replicated trials over several locations for yield performance. The best performing single crosses are identified for release as a variety or for use in the production of double cross hybrids.
- ✓ This method can evaluate only limited number of inbreds at a time, because inclusion of more inbreds in crossing increases the number of single crosses in such a way that their handling becomes difficult.
- ✓ Use of 50 inbreds, it will give rise to 50(50 - 1)/2 = 1225 single crosses.

Time of Testing:

- ✓ The testing of inbreds for general combining ability should be started from 3rd, 4th and 5th generation of selfing.
- ✓ This will help in retaining of inbreds with good combining ability and elimination of lines with poor combining ability.
- ✓ Some workers suggest that visual selection is effective in improving combining ability in early inbred generations.

3. Production of Hybrid Seed :

- ✓ After identification of superior inbred lines, the hybrid seed is produced. Any type of the hybrids can be developed.
- ✓ In case of single and three way cross hybrids, the rows of female and male parents are planted in 2: 1 or 3: 1 ratio (Planting ratios are crop specific).
- ✓ In case of three way cross hybrid, the single cross hybrid is used as female parent and inbred lines as male parent. In case of double cross hybrid, the rows of female and male parents are planted in 3: 1 or 4: 1 ratio.
- ✓ The seed production is carried out in isolation to prevent crossing with other compatible genotypes and maintain the high genetic purity.

Predicting Double Cross Performance:

- 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 use of single cross performance for the prediction of double cross performance has become a standard breeding procedure.
- 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) (C x D).
- Multilocational or multiseasonal testing is desirable to predict the performance of a double cross hybrid.
- The predicted yield is worked out as follow:

  $$\text{Predicted yield} = \frac{(A \times C) + (A \times D) + (B \times C) + (B \times D)}{4}$$

Exercise:

1. Write down procedure for development of hybrids.
2. Explain predicting double cross performance.

EXERCISE 13: STUDY OF GENERAL COMBINING ABILITY & SPECIFIC COMBINING ABILITY OF BREEDING LINES

Date:

Combining Ability:

- The concept of general and specific combining ability as a measure of gene action was first proposed by Sprague and Tatum (1942).
- Combining ability is the relative ability of a strain to transmit desirable performance to its progeny upon hybridization with other strains.

Types of combining ability

1) General combining ability (GCA)

- It is the average performance of a genetic strain in a series of cross combinations, estimated from the performance of F1's from crosses ***OR***
- It is the average ability of a parent to combine with a set of other parents in hybridization.

Characteristics of General combining ability

- GCA is related to parents only
- GCA effect of a parent (P) is calculated as **(G-M)**

  Where, G = mean value of crosses in which P is a common parent and

  M = General mean.
- GCA variance is attributed to additive and additive x additive interaction variance which is considered as fixable.
- The knowledge of GCA helps to evaluate the parents of broad genetic base.

2) Specific combining ability (SCA)

- It is the deviation in the performance of a specific cross combination from the performance predicted on the basis of general combining ability of the parents involved in the cross.
- It is used to designate those cases in which certain combinations do relatively better or worse than that would be expected on the basis of average performance of the parents involved in the cross.

Characteristics of specific combining ability

- SCA is related to crosses (hybrid) only
- The knowledge of SCA helps to identify the hybrids.
- High SCA may be due to good x good or good x poor or poor x poor combiners.
- If high SCA is due to high x high GCA effects, one can exploit the hybrid vigour in F_1.
- If high SCA is due to high x low GCA effects, such parents are used for development of sister lines which are used for the development of synthetic

varieties. Based on this knowledge, further breeding programme is to be formulated.

- If high SCA is due to low x low GCA effects, there are least chance of improving such parents.
- SCA effects of a cross is calculated as ($S - G_1 - G_2 - M$)

  Where, S = mean value of a specific crosses

  G_1 & G_2 = GCA effects of two parents involved in a cross, and

  M = General mean
- SCA variance is attributed to dominance genetic variance and additive × dominance and dominance × dominance interaction variance which are considered as non-fixable.
- Kempthorne (1957) defined GCA and SCA variances in terms of covariance of half and full sibs in a random mating population.
  - Progeny produced by GCA is half-sibs (H.S.), because

    $\square^2_{\square\square\square}$ = Cov. (H.S.) = $\frac{1}{4}$ $\square^2_{\square}$
  - Progeny produced by SCA is full-sibs (F.S.), because

    $\square^2_{\square\square\square}$ = Cov. (F.S.) – 2 Cov. (H.S.) = $\frac{1}{4}$ $\square^2_{\square}$

Methods to estimate combining ability:

1. Inbred –variety cross (Jenkins & Brunson , 1932)
2. Polycross (Tysdal *et al.,* 1942)
3. Diallel cross analysis (Griffing, 1956)
4. Line × Cross analysis (Kempthorne, 1957)

Utility of combining ability in plant breeding

- It helps to identify the parents having good potential to transmit desirable characteristics to their progenies.
- It helps to sort out the promising crosses for yield and its components.
- It also provides the information regarding the nature and magnitude of gene action involved in the inheritance of traits.
- It is valuable for adoption of efficient breeding programme.

Selection of parents based on GCA is more desirable as compared to that based on *per se* performance. Why?

- Parents are selected on the basis of per se performance such parents may be good but transmission ability to their progenies may or may not be good. While parents selected on the basis of GCA denotes good transmission ability to their progenies.
- In addition nicking ability of parents is also based on GCA. Therefore, parents selected on the basis of GCA are more desirable as compared to selection of parents on the basis of *per se* performance.

How Combining ability is superior over simple hybridization between any two parents at a time?

- The Knowledge of combining ability of parents helps to predict the performance of resulting hybrids and accordingly one can formulate the breeding programme, while in case of simple hybridization between any two parents; one can't formulate the breeding programme.

Relationship between combining ability and gene action:

1) **If only GCA variance is significant:** Additive gene action is important. In such case, pedigree method of selection may be effective and that should be followed.
2) **If only SCA variance is significant:** Non- additive gene action is important. In such cases, exploitation of heterosis in F1 should be followed.
3) **If both GCA and SCA variances are significant:** Both additive and non-additive gene actions are important. In such cases, parental mating approach or reciprocal recurrent selection by alternate selfing and inter mating should be followed which helps in isolation of desirable transgressive segregants in segregating generations.

Exercise:

1. Differentiate general and specific combining ability.
2. Enlist the methods to estimate combining ability.
3. Enlist the utility of combining ability in plant breeding.