

Practical Manual
B. Sc. (Hons.) Agriculture
Fundamentals of Plant Breeding
Course No.: ASPG2103
Credit: 3(2+1)
Semester: 3



Centurion
UNIVERSITY

Shaping Lives...
Empowering Communities...

Department of Genetics and Plant Breeding
& Seed Science and Technology
M. S. Swaminathan School of Agriculture
Centurion University of Technology and Management, Paralakhemundi
www.cutm.ac.in

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Certificate

Certified that this is a bonafide record of practical work done by

Mr./ Ms. _____

Regd. No. _____ in B. Sc. (Hons.) Agriculture degree programme,

*Course No. ASPG2103 entitled “Fundamentals of Plant Breeding” during 3rd semester of
the academic year 2023-24.*

Date:

Faculty In charge

Evaluation System
Fundamentals of Plant Breeding
Course No.: ASPG2103

Sl. No.	Marks distribution	Total marks	Marks obtained
1	Record		
2	Attendance		
3	Assignment		
4	Field work & Field Notebook		

Signature of Faculty In-charge

List of Experiments

Exp. No.	Date of Exp.	Date of Submission	Title	Page No.	Signature	Remark
1			Plant Breeder's kit			
2			Study of germplasm of various crops			
3			Study of floral structure of self-pollinated and cross-pollinated crops			
4			Different methods of emasculation techniques			
5			Emasculation and hybridization techniques in self-pollinated crops			
6			Emasculation and hybridization techniques in cross pollinated crops			
7			Consequences of inbreeding on genetic structure of resulting populations			
8			Study of male sterility system.			
9			Handling of segregation populations: Pedigree method			
10			Handling of segregation populations: Bulk method			
11			Handling of segregation populations: Back cross method			
12			Methods of calculating mean, range, variance, standard deviation, heritability			
13			Designs used in plant breeding experiment, analysis of Randomized Block Design (RBD)			
14			To work out the mode of pollination in a given crop and extent of natural out crossing			
15			Prediction of performance of double cross hybrids			

Experiment No. 01: Plant Breeder's kit

Date:.....

Aim: To know about the different instruments and their uses in plant breeding

The plant breeder has to follow selfing (control pollination) or artificial pollination for which certain instruments are required. The breeder's kit includes the following equipments.

1. **Fine pointed forceps:** Forceps are used for removing anthers from a bisexual flower bud. The delicate parts of flower are to be handled carefully during emasculation. They are also used for handling stamens/ anthers during artificial pollination.
2. **Fine pointed straight / curved scissor:** Small and medium sized are used for removing uncoated flower, buds, spikelets, branches and awns, they are also used for clipping sepals, petals when they enclose delicate floral parts.
3. **Needle with wooden handle:** Needle with wooden/plastic handle are used for opening the small buds and removing the anthers from bud they are also used for formation of better paper bags.
4. **Magnifying lens:** They are used for observing minute morphological feature such as bear's anthers, style, pollen etc. they are also used to a certain that stigmas of emasculated floral bud are from pollen grains/foreign bodies.
5. **Bags:** Bags butter paper, tissue paper, brown paper, polythene etc. of size used to cover the floral buds, branch, inflorescence after emasculation & pollination. The bags of paper size are used for selfing the floral buds to avoid in cross pollination and to maintain purity of seed. Cloth and wire bags are used to protect ear bead and fruit/spikes from buds attack, cloth/brown/polythene bags are used for staining the seeds.
6. **Petridish or watch glasses:** It is used for collecting anthers pollen grain for artificial pollination as well as for sterilization of equipment's.
7. **Fine hair brush:** Medium sized hair brush is used for transferring pollen grains to the stigmas of emasculated flower at the time of pollination.
8. **Alcohol of spirit bottle:** It is used for stretching needles, forceps and scission to acid contamination of foreign pollen during the emasculation and pollination.
9. **Wooden or iron stakes:** They are used for giving support to weak plant or their branches during hybridization.
10. **Labels:** labels of paper, aluminium or iron having different sizes are used as per requirement. They are mainly used to labels two flowers plant, plant row, plant or blacks etc. for identification.
11. **Field tags of coloured tracts:** Tags of various coloured threads are used to mark specific flower/fruit/spike/branches for identification then cabling is not possible.
12. **Field dairy / notebook:** It is used for recording day to day observation such as date of emasculation and pollination sourcing flowering harvesting etc., names of the parents and other details of experiments.
13. **Pencil & meter scales:** They are for recording observations on quantitative character such as height, spread of plants, and length of leaf and can heat etc.
14. **Balance:** Small weighting balance i.e. portable morsel balance i.e. used for recording the observations of weight of large number of samples quickly in the field only.

Things to do:

Draw neat labeled diagram of tools of plant breeder kit

Experiment No. 02: Study of Germplasm of Various Crops

Date:.....

Aim: To know about different types of germplasm of various crops and their utilization in Plant Breeding.

Germplasm

The germplasm of a crop may be defined as the sum total of hereditary material that is all the alleles of various genes, present in a crop species and its wild relatives. Therefore germplasm consist of the following 5 types of material:

1. Land races

These are primitive varieties, which had evolved over centuries or even million year through both natural and artificial selection, but without a systematic and sustained plant breeding effort. Genetic variation is present both within and between varieties. They are adapted to survive under unfavourable conditions and have low but stable alleles.

2. Obsolete varieties

These varieties were developed by systematic breeding effort, where once commercially cultivated, but are no more grown. They however, do have some desirable features .Examples wheat varieties K65, K68, Ph591, many NP series varieties etc.

3. Varieties in cultivation

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. They have been evolved through planned plant breeding, are ordinarily highly homogeneous and highly genetically variable.

4. Breeding lines

These are lines/ population developed in breeding programmes. They often contain valuable gene combination .These group includes nearly homozygous lines, mutants lines, lines derived from biotechnology programmes and new transgenic lines. These lines usually have narrow genetic base.

5. Special genetic stocks

The germplasm in this category includes those carrying genes mutations, chromosomal aberrations marker genes etc. These stocks are useful in genetic analysis and may find use in breeding programmes as well .These lines may have been obtained spontaneously or often induced artificially. An example of such a materials will be a set of monosomic or trisomic lines.

6. Wild forms and wild relatives

Wild forms are the wild species from which crop species are directly derived they are easy to cross with the concerned crop species. The wild relatives include all other species, which are related to the crop species by descent during the evolution .Both these groups are sources of valuable genes for insect and diseases resistant,tolerance to abiotic stresses like drought, cold, salinity etc, and even for quality traits and yield.

Things to do:

A) Germplasm utilisation

B) Write Some of the important germplasm collections of both National and International Institutions

S. No.	Institution	Location	Crop
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Experiment No.03: Study of Floral Structure of Self-Pollinated and Cross-Pollinated Crops

Date:.....

Aim: To study about the floral biology and reproductive behaviour of crop plants.

Objectives

1. To acquaint with the different parts of a flower and their function ex. Lodicules etc.
2. To study the opening and closing time of florets
3. To study the time of anther dehiscence and stigma respectively
4. To study the mode of pollination

Floral and reproductive morphology

The study of external morphology, more particularly floral morphology is necessary to describe a plant completely and to ensure presence or absence of different components of reproductive organs and also their arrangements. Without knowing the detailed reproductive morphology, it is not possible to certain about pollinating mechanism. it is also essential for planning and adopting correct hybridisation procedures during crop breeding program. The reproductive morphology includes.

- a) Flower with its complete description that is as presence of different addisonal structure like bracts, bracteoles and pedicel etc. or their modificationif any.
- b) Presence of different whorls like calyx, corolla, androecium and gynoecium
- c) Detailed description and modification if any of the different whorls
- d) Floral diagram and floral formula.

Floral biology

It includes anthesis of flowers that is opening of the floral bud and the time of its fertilization. The technique can be precisely determined only when the biology of the flowers is known. Floral biology of crop plants in quiet variable and it depends upon location, genotype, nutrient and environmental conditions like temperature, photoperiod and humidity.

Generally the floral biology is studied with regard to

- a) Flowering duration
- b) Panicle emergence
- c) Sequence of blooming in panicle
- d) Duration of blooming period
- e) Daily blooming
- f) Opening and closing of individual spikelet

- g) Time of anther dehiscence
- h) Viability of pollen grains and
- i) Stigma receptivity

Pollination system in crop plants

The manner in which florets are borne on the inflorescence determines the pollination mechanism. Some species have bisexual flower where both male and female reproductive parts present in same flower and when anthers are matured, they dehisce and shade the pollen on stigma. On the other hand several species have unisexual flowers which bears male and female flowers either on the same plant (monoecious, e.g. Maize) or on two different plant (dioecious, e.g. Papaya)

Similarly, receptivity of stigma is also determined by the presence of sugary substance on its surface. The shading of the pollen on the stigma takes place in different stages of bud opening. The stage, at which the pollen takes, depends upon the extent the cross or self-pollination.

Things to do:

1. Draw neat labelled diagram of Flower

2. Write different mechanisms promoting self and cross pollination

Experiment No. 04: Different Methods of Emasculation

Techniques

Date:.....

Aim: To know about the different methods of emasculation.

Emasculation:

It refers to the removal of unripe anther before dehiscence from a hermaphrodite flower turning the hermaphrodite bud/ flower into a pistillate one. In other words removal of immature anther from a bisexual flower is called emasculation. In bisexual flower, emasculation is essential to prevent self-pollination. In monoecious plants male flowers are removed (caster, coconut) or male inflorescence is removed (maize). In species with large flowers e.g. Cotton pluses hand emasculation is accurate and it is adequate also.

The main points related to emasculation are

1. Emasculation is relevant to bisexual (hermaphrodite) flowers only.
2. There are two main purposes of emasculation first to prevent self-pollination and second to prepare the female parent for crossing with the desired male parent.
3. In monoecious plants the male and female flowers are separate but present in the same plant/inflorescence such as in castor and maize. In castor male and female are present in the small inflorescence. In such case male flowers are removed from the inflorescence. In maize, male and female flower are in separate inflorescence in such case the male inflorescence is removed.
4. In dioecious species male and female flowers are present on the different plants such as papaya, date palm etc. In such case male plants are removed.

Methods of emasculation

- 1) Hand emasculation – (a) Forced open method (b) Clipping method
- 2) Emasculation with hot water.
- 3) Emasculation with cold water.
- 4) Emasculation with alcohol.
- 5) Suction method.
- 6) Chemical emasculation method.
- 7) Male sterility method.

1. Hand emasculation

This method is used with those crops which have relatively larger flowers such as cotton, okra, cowpea, field pea, black gram and green gram etc. It is also used in case of crops like wheat, barley, triticale, rice, rapeseed, mustard, brinjal, tomato, potato and several other crops. It is of two types such as

(a) Forced open method: This is the conventional method still used by the plant breeders, the corolla of the selected flower is opened with the help of forceps without mutilation of

gynoecium. The exact procedure varies from crop to crop i.e. sometimes corolla may be totally removed along with epipetalous stamens e.g. gingerly. In case of rice, the breeder is to separate the glumes with a pair of forceps in the early morning about 1-2 hours before undertaking pollination. Thus the six stamens are removed. It is generally done between 4 and 6 pm are day before anthers dehisce.

(b) Clipping methods: Emasculation and pollination may be facilitated however by cutting the ends of the florets. This technique is usually used in cereals like rice and wheat. In this method the top 1/3 portion of the spikelets are cut (slant) with scissors and the anthers are removed with forceps.

2. Emasculation with hot water

This method is used with those species in which flower size is very small and numbers of flowers in an inflorescence are too many such as sorghum, paddy and certain other grasses. Usually pollen grains are more sensitive to both genetics and environment fluctuation hence, hot water kills pollen grains without causing any damage to the gynoecium. The thermo flask of appropriate size with big mouth are used for carrying hot water to the field and the whole inflorescence is immersed in the hot water for a definite time before the anther dehiscence and prior to opening of the flower which varies from species to species. The temperature of water and duration of treatment vary from crop to crop. Temperature and duration of exposure required for emasculation in some crop plants are as follow.

Table 4.1: Temperature and duration of hot water treatment for emasculation in different crops

Name of the crop	Temperature requirement ($^{\circ}\text{C}$)	Duration of exposure (min.)
Rice	42-44	5-10
Sorghum	47-48	10
Smooth broom grass	46-48	3
Finger millet	52	2-2.5
Pearl millet	45-50	1-10

3. Emasculation with cold water

Cold water treatment like hot water kills the pollen grains without causing any damage to the gynoecium. It has been found effective in certain crops like rice and wheat for cold treatment one has to grow the plants in pots and treatment done in cooling chamber. Temperature and duration of exposure required for emasculation.

Table 4.2: Temperature and duration of cold water treatment for emasculation in different crops

Name of the crop	Temperature required ($^{\circ}\text{C}$)	Duration of exposure (hrs.)
Rice	0-6	10-12
Wheat	0-2	15-24

However, this method is lesser effective in killing pollen grains than hot water technique.

4. Alcohol treatment method

It is not a commonly used method and has been tried in very few crops. This method requires special skill because immersion for longer duration may cause damage to gynoecium. Duration of treatment is very important i.e. even a slightly prolonged period of treatment more than the recommended would reduce the seed set i.e. the female reproduction organs will also be killed. Dose and duration of alcohol treatment for emasculation.

Table 4.3: Dose and duration of alcohol treatment for emasculation in different crops

Name of the crop	Dose (%)	Duration (sec)
Lecurine	57	10
Sweet potato	55-57	8-10

5. Suction method

It is useful in species with small flower. This method has been used in rice. Anthers are removed through suction for which suction pump is used. A thin rubber or glass tube which is attached to the suction has been used to suck the stamens from the spikelets. The desired vacuum is created to suck the anthers without causing any injury to the gynoecium. Emasculation is done just before 1hr or immediately after opening of the spikelets. This method is lesser effective because considerable self-pollination, up to 10%.

6. Chemical emasculation method

It is a rapid method of developing male sterile line. Emasculation is done with same chemicals. The chemicals used for induction of male sterility are known as male gametocides or chemical hybridizing agents (CHA) which selectively kills the male gamete without affecting the female gamete. Examples of some CHAs are listed as below.

- 1) Ethrel- Rice
- 2) Sodium methyl arsenate- Rice
- 3) Zinc methyl arsenate- Rice
- 4) Malic hydrazide- Cotton, Wheat, Coriander
- 5) FIN-50- Rice
- 6) Trifluro methyl sulphonamide- Maize
- 7) Eribberellie acid- Coriander
- 8) Ethyl 4 flurooxanilate- Soyabean
- 9) Surf excel- Coriander
- 10) Ethyloxanilots- Wheat

7. Genetic emasculation

Genetic/ cytoplasmic – Genetic male sterility may be used to eliminate the process of emasculation. There is no need of emasculation where natural male sterility system operates. In general three types of male sterility operate in crop plants.

- a) **Genetic male sterility**- Every time 50% fertile plants are to be rogued out.
- b) **Cytoplasmic genetic male sterility**- This is usually preferred for commercial production of hybrid i.e. Maize, sorghum, onion, pearl millet, cotton and rice etc.

- c) **Cytoplasmic male sterility**- Used in the crop where seed is not economic part i.e. forage crop, sugarcane and potato etc.

Bagging

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

Crossing

Crossing refers to artificial pollination between selected genotypes etc. in order to

1. Create genetic variability in a population.
2. Combine genes from various sources such as germplasm lines wildspecies etc.
3. Develop hybrids – Generally the pollen grains collected from a desired male parent should be transferred to the emasculation flower. This is normally done in the morning hours during peak anthesis. The flowers are bagged immediately after artificial crossing.

Tagging

The flowers are tagged just after bagging. The tags are attached to the inflorescence or to the flower with the help of a thread containing the following information.

1. Date of emasculation (DOE)
2. Date of pollination (DOP)
3. Percentage i.e. parents involved in the crossing.
4. No. of flower emasculated.
5. Name of the breeder etc.

Things to do:

1. Draw neat labelled diagrams of different emasculation methods

Experiment No. 05: Study of Floral Structure, Emasculation and Hybridization Technique in self-pollinated crops: Rice

Date:.....

Aim: To study floral morphology, emasculation, artificial selfing and crossing in rice plant.

Rice:

- Common name - rice
- Scientific name - *Oryza sativa*
- Family - poaceae
- Sub-family - orizoideae
- Chromosome no. - $2n=24$
- Origin - South and South-East tropical Asia
- Related species - *O. glaberrima*, *O. perennis*

Rice is a staple food of more than 60% of the world's population. Maximum area under rice is in Asia. Among rice growing countries, India has the largest area of about 40.2 million-hectare followed by China and Bangladesh.

Floral morphology

- ❖ **Inflorescence:** The inflorescence is called Panicle which is a group of single flowered spikelets born at the end of vegetative shoot. It is branched with different no. of spikelets attached to small rachillae. Primary rachis has hollow internodes and solid nodes. The no. of branches on the rachis, arrangement of spikelets and size of grains vary from variety to variety.
- ❖ **Spikelets:** The spikelets are single flowered, each with a short stalk. The rice spikelets or florets consist of two empty sterile glumes, two fertile glumes, two fleshy lodicules, six stamens in whorls and a pistil having a single central ovule, a short slender style and a bifurcated feathery stigma. The spikelet is connected to the rachillae at its base by a ball and socket arrangement.
- ❖ **Glumes:** There are two small glumes at the base of each spikelet.
- ❖ **Lemma:** The lemma is large and hairy. It is awned 5-nerved structure.
- ❖ **Palea:** The palea is hairy, smaller than the lemma and is present opposite to it. It is three-nerved structure. After maturity, the lemma, the palea and the glumes remain attached on the seed as a cover called husk.
- ❖ **Flower:** The flower is bisexual, zygomorphic and bracteate.
- ❖ **Perianth:** The two-minute scales like structures present at the base of florets are called the lodicules. They are thick and fleshy.
- ❖ **Androecium:** The androecium consists of six stamens arranged in two whorls whose filaments are short in earlier stage.
- ❖ **Gynoecium:** The gynoecium is monocarpellary and has a superior ovary, with two feathery stigmas on a style. Maximum receptivity of stigma is during the first 3 days after opening of spikelet and then is gradually lost after 7 days.
- ❖ **Anthesis:** Rice is a self-pollinated crop, with 0.1 to 4 per cent outcrossing. The flower may open from 7 a.m. to 4 p.m., depending upon the season. Most of the

flowers start opening at the apex and the flowering proceed downward in the panicle, but in the branches, it is not strictly so.

Emasculation

Breeders used the conventional method of emasculation. In which the glumes are separated with the help of a pair of forceps. In early morning that is 1-2 hours before pollination all the six stamens are removed. Emasculation should be completed usually 2 hours before the time of anthesis. clipping across anthers just above the attachment of filaments will remove most of the anthers. In general emasculation is completed in afternoon and pollination done in next day morning.

Several methods have also been used for emasculating rice.

- 1) **Hand emasculation:** Normally the individual panicle which is 50-60% emerged from the boot leaf is selected for emasculation. Emasculation is done usually with hand emasculation or vacuum method.
- 2) **Hot water treatment:** It is also possible at 40-44° C for 10 minutes before anthesis prior to opening of the flower.
- 3) **Cold water treatment:** It is also possible at 0-6° C for 15-24 hrs.
- 4) **Clipping method:** This method of emasculation one third to half of each of remaining 7-10 florets are to be clipped across the anthers to expose the anthers for easy emasculation.
- 5) **Forced open method:** In this method the glumes are separated with a pair of forceps, in the early morning that is 1-2 hours before pollinations and all the 6 anthers are removed carefully.
- 6) **Genetic emasculation:** Male sterility and self incompatibility

The emasculated panicles are covered with butter/ brown paper bags to prevent the out crossing from undesirable pollen source.

Pollination

Collect the pollens just before anther dehiscence. To apply pollens, remove butter paper bags and the anthers from the desired male parents are shaken over the emasculated florets. If pollens are not available in plenty then repeat the above process. If two panicles on the same plant are to be pollinated by the same male parent, they are brought together by wrapping the two culms and are tied with a clip. The other bag is also placed over both panicles.

Things to do:

1. Draw a neat labeled diagram of Rice spikelet with floral formulae

2. Emasculate and pollinate one of the self-pollinated crop and Attach the photo of same

Experiment No. 06: Study of Floral Structure, Emasculation and Hybridization Technique in cross pollinated crops: Maize

Date:.....

Aim: To study about floral morphology, emasculation, artificial selfing and crossing in maize plant.

Maize:

Common name : Maize
Scientific name : *Zea mays* L.
Family : Poaceae
Sub family : Poideae
Chromosome number : $2n=20$
Origin : Southern Mexico and Central America
Related species : *Zeamexicana* (Annual teosinte)

Zeaperennis (Perennial tetraploid) &

Tripsachum (gamma grass)

Floral biology

Inflorescence:

Maize plant is monoecious. The male or staminate flowers are found, on the terminal inflorescence known as tassel whereas, female flowers are on the lateral branches called ear shoots (cobs).

(a) Male inflorescence

The male flowers are born on the terminal inflorescence known as tassel. The staminate or tassel containing male flowers which is continuation of the main axis always terminal in position and therefore only one tassel is found per stalk. Primary, secondary and tertiary branches are also present in a tassel. The spikelets are usually arranged in pair one being sessile and the other being pedicelated. Paired spikelets are arranged in several rows on the main axis and in two rows on the lateral branch. These spikelets are similar in structure, one is borne on a short pedicel and other is sessile. Each spikelet consists of a pair of glumes that comprises of two florets, each of which is enclosed by a lemma and palea and has two lodicules, three stamens and rudimentary pistil.

(b) Female inflorescence

The pistillate inflorescence or female inflorescence which develops into an ear and they are born at the side of plants into an axis of the leaves, about midway of the stalk on a short branch as shank, the shank consist of modifying leaves enclosing ears and are collectively known as husk. The sessile spikelets are present in pair on a rachis. Due to this, each cob contains even number of kernel rows. The pistillate spikelets are born on a thick axis called a cob. Within each spikelet, two flowers are present one is abortive and other bears

stigma. A pair of much reduced glume is also present. The lower flower is reduced to form lemma and palea. Rudimentary lodicules may also be present. The pistil is tricarpeled, out of which two carpels extend in the thread like structure to form silk and third forms ovule, the silk is the modification of style. Fresh silks are receptive to pollen throughout their length. Since only one flower is fertile usually an even number of rows are present in the ear.

Anthesis

- ❖ The tassel blossoming starts from just below and before the tip in the central axis, then it progresses upward and downward simultaneously.
- ❖ Blossoming may be finished by noon and a tassel takes up to 14 days to complete flowering.
- ❖ Dispersal of pollen may begin about 3 hours after sunrise and continue for 1 to 3 hours.
- ❖ Pollen shedding begins 1-3 days before emergence of the silk from the same plant and continues up to 3 or 4 days.
- ❖ Single tassel produces 250,000 pollen grains. Pollen remains viable for 12-18 hours under favorable conditions. Pollens are highly susceptible to heat and desiccation.
- ❖ Silks become receptive as soon immediately after emerging from the ear husk. It may require up to 5 to 6 days for all silks to emerge from an ear. Receptivity up to 14 days after emergence has been reported.

Modes of pollination

Maize is generally protandrous, that is, the male spikelets mature earlier than the female spikelets. Because of monoecious plant habit and protandrous nature of anthers, the maize is considered a highly cross-pollinated crop. The pollen is generally dispersed by wind. There may also be a chance of about 5 per cent self-pollination.

Selfing and crossing techniques

Selfing

- ❖ At the time of flowering, right plant must be selected to cover the ear shoot.
- ❖ Ensure the stage of cob is such that the silk emerges after one or two days.
- ❖ Short bags are placed over each ear shoot.
- ❖ Tassel of the same plant is also covered with tassel bag (craft paper of size 7.5 cm x 16 cm) for the collection of the pollen.
- ❖ Check the ear shoot daily. If it is at the right stage, then in the morning hours the tassel bag of the same plant is lifted and dusted on the cob.
- ❖ Bag the spadix with tassel bag and repeat the process in the next morning.
- ❖ Put the tag and label it.

Crossing:

- ❖ A tassel produces its utmost quantity of pollen in the second and third days of dehiscence.
- ❖ The tassel bag is placed over the tassel of the male parent. The bag is tied by a paper clip or a staple.

- ❖ When the ear shoot is at the right stage of the female parent and the tassel of the male parent also has abundance of pollen grains, the tassel bag is lifted and dusted on the ear shoot and the ear shoot bag is replaced by the same tassel bag to supply maximum amount of male parent pollen grains.
- ❖ The bag should again be fastened by paper clip.
- ❖ The tassel bag should remain over the ear shoot until harvest.
- ❖ When more than one ear on a plant is pollinated, seed set on the lowest ear is most reliable, if pollinated one day before the ear at higher node. Lower ear has least chances of contamination.
- ❖ Label the tassel bag with graphite pencil properly indicating male parent, date of pollination etc.

Precautions:

- ❖ Bagging of the tassel should be done in previous night to avoid contamination.
- ❖ Programme name & date of pollination is to be mentioned on the tassel & ear bag.
- ❖ Pollination should be carried out one day after tasseling.
- ❖ Pollination should be completed within 4 hrs. after tasseling.
- ❖ Pollination should be completed within 4 hrs. after removal of tassel.
- ❖ Pollination should be completed within a week.

Things to do:

1. Draw a neat labeled diagram of Maize inflorescence with floral formulae

- 2. Emasculate and pollinate one of the cross-pollinated crop and Attach the photo of same**

Experiment No. 07: Consequences of Inbreeding on Genetic Structure of Resulting Populations

Date.....

Aim: To know the consequences of inbreeding on genetic structure of resulting population.

Inbreeding

Inbreeding is the production of offspring from the mating or breeding of individuals or organisms that are closely related genetically.

Inbreeding or consanguineous mating is the mating between individuals related by decent or ancestry.

Inbreeding results in homozygosity which can increase the chances of offspring being affected by deleterious or recessive traits. It can result in purging of deleterious alleles from a population through purifying selection. It is a technique used in selective breeding.

OBJECTIVES OF INBREEDING

1. Increase the proportion of homozygosity in a population.
2. Elimination of genetic load from the population because unfavourable recessive allele become homozygous and are eliminated from the population.
3. Inbreeding is accompanied with reduction in vigor or reproductive capacity.

Effects of inbreeding

Inbreeding is generally accompanied with a reduction in vigour and reproductive capacity that is fertility. There is general reduction in the size of various plant parts and in yield. In many species, harmful recessive alleles appear after selfing; plants or lines carrying them usually do not survive. The effects of inbreeding may be summarized as under,

1. **Appearance of Lethal and sub lethal Alleles:** Inbreeding results in the appearance of lethal sub lethal and sub vital characteristics, such characteristics include chlorophyll deficiencies e.g.- Albina, chloric, etc. Generally plants carrying such characteristics cannot be maintained and are lost from the population.
2. **Reduction in vigour:** There is general reduction in the vigour of the population plants become shorter and weaker because of a general reduction in the size of various plants parts.
3. **Reduction on reproductive ability:** The reproductive ability of the population decreases rapidly many lines reproduce so poorly that they can't be maintained. In most of the crops only a small number of inbred lines have enough fertility to be easily maintained and to be useful in breeding program.
4. **Separation of the population into distinct line:** The population rapidly separates into phenotypically distinct lines. This is because of an increase in homozygosity due to which there is a random fixation of various alleles in different lines therefore the lines differ in their genotype and consequently phenotype. This leads to an increase in the variance of a population as a whole.

5. **Increase in homozygosity:** Each line becomes increasingly homozygous following inbreeding. Consequently, the variation within a line decreases rapidly. Ultimately after 7th to 8th generations of selfing the lines become almost uniform since they approach complete homozygosity. The lines, which are completely homozygous due to continued breeding and are maintained through close inbreeding, are known as inbred lines or inbreds. Inbred lines have to be maintained by strict breeding preferable by selfing in order to keep them homozygous.
6. **Reduction in yield:** Inbreeding generally leads to a loss in yield. The inbred lines that are able to survive and be maintained yield much less than the open pollinated varieties from which they were derived.

Inbreeding depression

Inbreeding depression may be defined as the reduction or loss in vigour and fertility as a result of inbreeding.

Inbreeding depression = $[(F_1 - F_2)/F_1] \times 100$ where F_1 and F_2 are the mean values of F_1 and F_2 progenies, respectively of the same cross for a given character.

Degrees of Inbreeding Depression:

High Inbreeding depression- Alfaalfa, carrot

Moderate Inbreeding depression- Maize, Jowar, Bajra

Low Inbreeding depression- Onion, Cucurbits, Rye, Sunflower

Lack of Inbreeding depression- Selfpollinatedsps.

Things to do:

1. Numerical expression of Genetic consequences of inbreeding in both self and cross

Pollinated crops

Experiment No. 08: Study of Male Sterility System

Date.....

Aim: To study about different types of male sterility system and their utilization in plant breeding

Male sterility: it can be defined as absence or non-function of pollen grains on plant or incapacity of plants to produce or release functional pollen grains. It acts as an important out breeding device which promote the heterozygosity. Male sterility can be resulted from the action of nuclear gene or cytoplasmic gene or both.

Classification of male sterility

Male sterility is mainly categorized into 4 main divisions they are:

1. Genetic male sterility- (a) TGMS (b) PGMS (c) Transgenic GMS
2. Cytoplasmic male sterility
3. Cytoplasmic genetic male sterility
4. Chemically induced sterility

1. Genetic Male Sterility

The pollen sterility which is caused by nuclear genes is termed as genetic or nuclear male sterility. It is usually governed by a single recessive gene *ms* or *s* with monogenic inheritance but dominant gene governing male sterility is known, e.g. safflower. This is two-line system i.e. A line (genetic male sterile line) and B line (heterozygous fertile line). A male sterile line may be maintained by crossing it with heterozygous male fertile plant. Such a mating produces 1:1 male sterile and male fertile plants

Types of genetic male sterility

Genetic male sterility is sub divided into the followed groups:

(A) TGMS (Temperature Sensitive Genetic Male Sterility)

In this type of GMS complete male sterility is produced by the *ms* gene at higher temperature (e.g. 23.3° c or higher for rice TGMS line *pie AI645*) but at temperature below this critical point there is normal fertility this type of genetic male sterility is being used to develop hybrid rice in china. Two rice TGMS (UPRI 94-140 TGMS & UPRI 95-167 TGMS) have been isolated as spontaneous mutants and registered as germplasm).

(B) PGMS (Photoperiod sensitive genetic male sterility)

In case of PGMS expression of *ms* gene is drastically affected by the prevailing photoperiod provide the temp below a critical range (e.g. 23-24° c for rice PGMS) within this temperature range complete sterility in rice is obtain grain in under long day condition (day length more than 13 hr. 45 min) but under short day condition almost normal fertility is

obtained. For example, genetic male sterile lines 5047S and W6154S have same ms gene from Nong-Keng58S. Line 5047S shows PGMS, while W6154S shows TGMS.

(C) Transgenic genetic male sterility (TrGMS)

A gene introduced in gamete of an organism by recombinant DNA technology or genetic engineering is called transgene. Many transgenes have been shown to produce genetic male sterility which is dominant to fertility. In such cases, it would be essential to develop effective fertility restoration system for their use in hybrid seed production.

- ❖ *Barnase* / *Barstar* system is a good example of transgenic male sterility.
- ❖ The *Barnase* gene of *Bacillus amyloliquefaciens* encodes an RNase. When *Barnase* gene is driven by TA29 promoter, it is expressed only in tapetum cell causing their degeneration. Transgenic tobacco and *Brassica napus* plant expressing *Barnase* were completely male sterile.
- ❖ Another gene, *Barstar*, from same bacterium encodes a protein, which is highly specific inhibitor of *Barnase* RNase.
- ❖ Therefore, transgenic plants expressing both *Barnase* and *Barstar* are fully male fertile.

2. Cytoplasmic Male Sterility

When pollen sterility is controlled by cytoplasmic gene known as Cytoplasmic Male Sterility. CMS is the result of mutation in mitochondrial genome (mtDNA) e.g. cms-T of maize, *Ogura* CMS of brassica or cytoplasmic genome e.g. tobacco, barely, sorghum and rapeseed.

Usually the cytoplasm of zygote comes primarily from the egg cell and due to this progeny of such male sterility plants would always be male sterility. The male sterile line is maintained by crossing it with pollinator strain (recurrent parent) in backcross, since the nuclear genotype of the pollinator is identical with that of the new male sterile line. Such a male fertile line is known as maintainer line or B line and male sterility line is also known as A line. CMS is not influenced by environmental factor.

3. Cytoplasmic Genetic Male Sterility

When pollen sterility is controlled by both cytoplasmic and nuclear gene known as CGMS (Nucleoplasmic male sterility). Jones and Davis first discovered this type of male sterility in 1994 in cotton.

The system includes A, B and R line. A line is a male sterile line, B is similar to 'A' in all features but it is a male fertile and R is restoring line; it restores the fertility in the F₁ hybrid. Since B line is used to maintain the fertility and is also referred as maintainer line. The fertility restorer gene R is dominant and found in certain strain of species that is Maize, Jowar, Bajra, Sunflower, Rice, Wheat etc.

4. Chemically induced male sterility

CHA is a chemical that induces artificial, monogenic male sterility in plants so that they can be effectively used as female parent in hybrid seed production. It is also called as

male gametocide, pollenicide, male sterilants, selective male sterilants, pollen suppressants, androicide etc.

First report was given by more and Nulum in the year 1950. They included male sterility in maize using Maleic Hydrazide (MH). NC Rac (1985) used in a single term chemical hybridizing agent (CHA). Examples of some CHAs are listed as below.

- 1) Ethrel- Rice
- 2) Sodium methyl arsenate- Rice
- 3) Zinc methyl arsenate- Rice
- 4) Malic hydrazide– Cotton, Wheat, Corionder
- 5) FIN-50– Rice
- 6) Trifluro methyl sulphonamide– Maize
- 7) Eribberellie acid– Corionder
- 8) Ethyl 4 flurooxanilate– Soyabean
- 9) Surf excel– Corionder
- 10) Ethyloxanilots– Wheat

Things to do:

1. Genetic male sterility – Maintenance of male sterile line

2. Transfer of cytoplasmic male sterility to a new strain

3. Inheritance of cytoplasmic genetic male-sterility

4. Production of double cross maize hybrids using CGMS

5. Transgenic Genetic male sterility

Experiment No. 09: Handling of segregation populations: Pedigree method

Date.....

Aim: To know about how to handle segregating population of pedigree method

Mass selection and pure line selection cannot be applied to segregating population E.g. F_2 , F_3 etc. because in these methods selection made from the existing variability present in the population. However, when there is limited genetic variability in a species than hybridization acts as an important tool to create additional genetic variability for further improvement of the species. The methods generally used for handling of segregation generation may be grouped into following three categories.

- a) Pedigree Method
- b) Bulk Method and
- c) Back Cross Method

The objectives of all these methods are to

- 1. Develop pureline
- 2. Develop new varieties.
- 3. Develop inbred line
- 4. Improve specific character of a well-adapted variety for which it is deficient.

Pedigree method

In pedigree method, individual plants are selected from F_2 and the subsequent generation and their progenies are tested. During the entire operation, a record of the entire parent's offspring relationship is kept, is known as pedigree record. The selection of individual plant is continued till the progenies show no segregation. At this stage, selection is done among the progenies, because there would be no genetic variation within progenies.

Things to do:

1. Schematic representation of pedigree method along with description

2. Maintenance of Pedigree record

Experiment No. 10: Handling of segregation populations:

Date.....

Bulk method

Aim: To know about how to handle segregating population of bulk method

Bulk population method

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

Things to do:

- 1. Schematic representation of bulk method along with description.**

Experiment No. 11: Handling of segregation populations: Back cross method

Date.....

Aim: To know about how to handle segregating population of backcross method

Backcross method

The plan of back cross method depend upon whether the gene being transferred is recessive or dominant. The plan for transfer of a dominant gene is quite simple than for recessive gene.

Requirements of a backcross programme

1. Existence of a good recurrent parent variety which requires improvement is some qualitatively inherited character or a quantitative character with high heritability.
2. A suitable donor parent must be available possessing the character or characters to be transferred in a highly intense form.
3. High expressivity of the character under transfer through several back crosses in the genetic back ground of the recurrent parent.
4. The character to be transferred must have high heritability preferably determined by one or few genes.
5. Simple testing technique for detecting the presence of the character under transfer.
6. Recoveries of the recurrent genotype in a reasonable number of back cross generations.

I. Transfer of Dominant Gene

Let us suppose that a high yielding and widely adopted variety 'A' is susceptible to stem rust (rr) and another variety 'B' is poor yielding but resistant to stem rust (RR) i.e. dominant to susceptibility. In this back cross programme rust resistance trait is transfer from donor parent (B) into a recurrent parent (A).

- A) Give the schematic representation of backcross method for transfer of dominant gene from variety B to an otherwise improved variety A**

II. Transfer of Recessive Gene:

When rust resistant is due to a recessive gene, all the backcross cannot make one after other. After the first backcross and after every two backcrosses F_2 must be grown to identify the rust resistant plants. The F_1 and the back cross progenies are not inoculated with rust because they would be susceptible to rust. Only F_2 is tested for rust resistant.

Things to do:

- 1. Give the schematic representation for the transfer a recessive gene governing rust resistance.**

Experiment No.12: Methods of calculating mean, range, variance, standard deviation and heritability

Date.....

Aim: To study method of calculating mean, range, variance standard and deviation and heritability

Introduction

Continuous variation present in the population is partly heritable and partly non-heritably. The non-heritable components arise from the influence of external environment, whereas heritable component depend on genes of many loci working together. The continuous variation cannot be analysed based on class frequencies alone but analysis of biometrical parameters is essential, various estimates to analyse continuous variation include arithmetic mean, mode, median, range, and standard deviation, standard error, co-efficient of variation, variances and covariance.

Arithmetic Mean

It is defined as the sum of all observations in a sample divided by their number (or) it is the average value of a set of observations (or) a data.

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

Where, \sum = Summation, X = no of observations and N = no of observations

Mode: Middle frequent values in a population.

Median: Middle value in an array of observations.

Standard Deviation (SD)

It is the square root of arithmetic mean of squares of all the deviations measured from the mean. It is also the square root of variance and calculated using the formula

$$\sigma = \sqrt{\frac{\sum [x - \bar{x}]^2}{n}}$$

Where, σ = standard deviation of population

\sum means "sum of,"
 X = each value in the data set,
 \bar{X} = mean of all values in the data set,
 N = number of values in the data set.

Variance

It is defined as the average of square deviation of all the individual observations from the mean (or) it is the difference between the sums of squares of all the observations in a sample from its mean divided by the degrees of freedom.

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

***Note- The important change is to divide by N-1 (instead of N, in case of population variance σ^2) when calculating a Sample Variance (S²).**

Standard Error (SE)

It is defined as the measure of difference between the sample mean and the populations mean (H). it is a measure of uncontrolled variation present in sample (\bar{x}). It is the ratio of standard deviation to the square root of no. of observations. It is customary to denote any sample in turns of its mean \pm SE.

$$SE = \frac{SD}{\sqrt{N}}$$

Co-efficient of Variation (CV)

All the above measures are absolute being expressed in terms of units in which they are recorded. A measure of variation which is independent of its unit of measurement is co-efficient of variation. It is the ratio standard deviation of a sample to its mean expressed as percentage (%).

$$CV = \frac{SD}{\bar{x}} \times 100$$

Heritability

The ratio of genotypic to phenotypic or total variance is known as heritability. It is generally expressed in per cent (%). Thus heritability is the heritable portion of phenotypic variance.

$$\text{Heritability (H)} = \frac{V_g}{V_p} \times 100 = \frac{V_g}{V_g + V_e} \times 100$$

Where, V_g, V_p and V_e specifies the genotypic variance, phenotypic variance and environmental variance respectively.

Things to do:

- Problems on Mean, range, variance, standard deviation and heritability

Experiment No. 13: Designs used in plant breeding experiment, analysis of Randomized Block Design

Date.....

Aim: To provide an introduction to experimental design including different layouts and considerations for statistical inference of RBD

Experimental design is the process of choosing treatments, responses, and controls, defining experimental and sample units, and determining the physical arrangement, or layout, of experiment units. Experiments should be designed to control error and give reasonable assurance that the differences between treatments will be detected.

Principles of experimental designs

Principles of experimental designs help in reducing the experimental error and thus make the experiment more efficient. There are three basic principles of experimental designs are briefly discussed as follow.

1. **Replication:** The repetition of treatment under investigation is known as replication. Increase in replication increases the precision by reducing the error to a greater extent.
2. **Randomization:** The allocation of treatments to different plots in an experiment by a random process is known as randomization of treatments. Randomization provides equal chance to all treatments for being allotted to more fertile plot as well as to less fertile plot.
3. **Local control or error control:** The principle of making use of greater homogeneity in a group of experimental unit for reducing experimental error is called local control. The fertility of the field may be of two types (a) Gradient (can be reduced by dividing the field into homogeneous block or replication and (b) Patches (can be minimized by randomization of treatments within the block).

Experimental designs which are commonly used in plant breeding and genetics includes

1. Simple Experimental Designs
2. Augmented Design
3. Completely Randomized Design (CRD)
4. Randomized Block Design RBD)
5. Latin Square Design (LSD)
6. Split Plot Design (SPD)
7. Lattice Design (LD)

Randomized Block Design (RBD)

The variability present in a population is of polygenic nature and these polygenic variations are three types viz. phenotypic, genotypic and environmental

The statistical procedure which separate (or) splits the total variation into different component is called analysis of variance or ANOVA .

ANOVA is useful in estimating the different components of variance. It provides basis to the test of significance and it is carried out only with replicated data obtained from standard statistical experimental results. The different components of variance are calculated as suggested by Lush in 1940.

ANNOVA Table

Source	Df	Ss	Mss	F-value calculated
Replication	(r-1)	Rss	$Msr = Rss / r-1$	Msr/Msc
Treatment	(t-1)	Trss	$Mst = Trss / t-1$	Mst/Msc
Error	(r-1)(t-1)	Ess	$Msc = Ess / (r-1)(t-1)$	
Total	(rt-1)	Tss		

F (calculated) is compared with F (table) value by looking at the F-table for replication (r-1) & error df values (r-1)(t-1). If the calculated F value is greater than F(table value) then it is significant.

Genetic variance

It is the inherent variation which remains unaltered by the environment. It is variation due to genotypes. It is denoted by V_g & is calculated using the formula

$$V_g = Mst - Mse/r$$

Where, Mst = mean sum of squares of treatment

Mse = mean sum of square of error

r = number of replications

Co-efficient of variation

- (1) **Phenotypic co-efficient of variation:** It is defined as the ratio of phenotypic standard deviation to the mean, expressed as % and can be calculated using the formula.

$$PCV = \frac{\sqrt{V_p}}{\bar{x}} \times 100$$

- (2) **Genotypic coefficient of variation:** GCV is the ratio of genotypic standard deviation to the mean expressed as % and is calculated using the formula

$$GCV = \frac{\sqrt{V_g}}{\bar{x}} \times 100$$

- (3) **Environmental coefficient of variation:** It is the ratio of environmental standard deviation to the mean expressed as % and is calculated using the formula

$$ECV = \frac{\sqrt{V_e}}{\bar{x}} \times 100$$

Where, V_g , V_p and V_e are the genotypic, phenotypic and environmental variance and \bar{x} is mean.

Result Interpretation

- A. GCV And PCV** are classified as suggested by Sivasubramonium&Madhavarmenon in 1973 as
- < 10% Low
 - 10-20% Moderate
 - > 30% High

Interpretation of Coefficient of variance

- ❖ **If, GCV > PCV**, it means little influence of environment on the expression of character. Selection for improvement of such character would be rewarding.
 - ❖ **If, PCV > GCV**, it indicates that apparent variation is not only due to genotype but also due to environment. Selection for improvement of such traits sometime may be misleading.
 - ❖ **If, ECV > PCV & GCV**, it indicates that environment playing significant role in expression of such characters. Selection for improvement of such traits would be ineffective.
- B. Heritability:** Jonnesenet. *al.* (1995) categorized heritability as
- <30% = Low
 - 30-60% = Moderate
 - >60% = Higher

Other scale of broad sense heritability

- >40% = Low
- 40%-60% = Medium
- 60%-80% = High
- >80% = Very High

Interpretation of heritability

- ❖ **If it is high:** Means characterless influenced by environmental effect, the selection for improvement of such character may not be useful because broad sense heritability includes both fixable (additive) and nonfixable (dominance and epistasis) genetic variance.
- ❖ **If it is low:** it reveals that character is highly influenced by environmental effect, and the genetic improvement through selection would be difficult due to masking effect of environment on the genotypic effect.

C. Genetic Advance: It is a measure of genetic gain under selection. It refers to the improvement of near genotype value of selection lines and families over the base population.

$$GA = (K) (H) (SDP)$$

Where,

H=heritability

SDP=phenotypic standard deviation

$$K = \text{selection differential} = (k) (v_p) (1/2V_g/V_p)$$

$$K = x_s - x_o$$

Where- x_s = mean phenotypic value of selected plant population

x_o = mean phenotypic value of parental plant

Interpretation of Genetic Advance (GA)

- ❖ **If GA is high:** Means character is governed by additive gene action and selection will be rewarding for improvement of such character.
- ❖ **If GA is low:** It indicates that character is governed by non-additive gene action and Heterosis breeding may be useful

Interpretation of Both Heritability and genetic advance:

- a) **High heritability and High genetic advance** means heritability mainly due to additive gene effect and hence selection may be effective.
- b) **High heritability and low genetic advance** is indicative of non-additive gene action and hence selection may not be rewarding.
- c) **Low heritability with high genetic advance reveals** that character governed by additive gene. Selection may be effective in this case.
- d) **Low heritability and low genetic advance** shows that character is highly influenced by environment and so selection would be ineffective.

Things to do:

- **Problems on calculation of different components of variance in RBD and interpret your results.**

Experiment No. 14: Work out the mode of pollination in a given crop and extent of natural out crossing

Date.....

Aim: To know about the mode of pollination in given crop and outcrossing determination

Determination of mode of pollination

Mode of pollination in a given crop can determine by following ways.

- ❖ The first step in determining the mode of pollination of a species is to critically examine its flowers. Mechanisms like dioecy, monoecy, protogyny, protoandry 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 seed set in isolation proves the species to be cross pollinated. However, setting of seeds is only indicative of self-pollination.
- ❖ Finally the effect of selfing (inbreeding) on the vigour of plant 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 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 recognisable seed or seedling character, while the other strain has the recessive form of the character. The two strains are 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 the seed 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 locations. For example, in study on safflower, the estimates the outcrossing in different varieties grown in the same year at the same location ranged from 0-8.7 %. 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. In view of this, the information on the amount of cross pollination in many crops may be regarded as incomplete.

Things to do:

1. Identify self and cross pollinated plants based on flower morphology present in your locality and write morphological descriptors used for identification.

Experiment No. 15: Prediction of performance of double cross hybrids

Date.....

Aim: to know how to predict the performance of double cross hybrid

In a double cross hybrid, four inbred parents are involved. The performance of a double cross hybrid can be predicted through any of the four methods indicated by **Jenkins (1934)**. Starting with the simplest procedure taking four inbreds namely A, B, C and D, these methods are:

1. **Top cross testing** (one cross per inbred) to know the breeding value to each of the four inbred (total four top crosses per double cross i.e. $A \times OPV$, $B \times OPV$, $C \times OPV$ and $D \times OPV$, where OPV= open pollinated variety).
2. **Mean of the four non-parental single cross** involved in $(A \times B) \times (C \times D)$ double cross viz. $(A \times C)$, $(A \times D)$, $(B \times C)$, and $(B \times D)$ (total 4 non parental single crosses per double cross).
3. **Average yield performance of all possible six single crosses** $[n(n-1)/2]$ namely $A \times B$, $A \times C$, $A \times D$, $B \times C$, $B \times D$ and $C \times D$ (total six single crosses per double cross).
4. **Average progeny performance of each inbred** can be determined by the mean performance of each inbred in all possible single crosses of $A \times B$, $A \times C$ and $A \times D$ will determine the average breeding value of the inbred A. Similarly, the mean of $A \times B$, $B \times C$, and $B \times D$ will indicate the potential of the inbred B and so on (total 12 crosses per double cross).

The available evidence shows that the method (b) i.e., mean performance of non-parental single crosses is the, most adequate and effective. Since there is a close correspondence between predicted and realized yields of double crosses in maize. Fortunately, the total number of crosses required to be sample per double cross is also the minimum, thus greatly the testing programme.

- ❖ **All possible single crosses(in a Diallel mating)= $n(n-1)$**
Where n=number of inbred lines.
- ❖ **All possible single crosses excluding reciprocal crosses = $n(n-1)/2$**
- ❖ **Total number of three way crosses= $n(n-1)(n-2)/2$**
- ❖ **Total number of double cross = $n(n-1)(n-2)(n-3)/8$**

Things to do:

- **Problems on prediction of double cross hybrids performance**

