PBG.202 METHODS OF PLANT BREEDING 1+1

THEORY

Reproductive and pollination systems in crop plants – Contrivances to aid self and cross pollination – Incompatibility - Sterility - Apomixis and their classification.

Centre of origin – Domestication of crop plants – germplasm, plant genetic resources – conservation and utilization – biodiversity.

Basic biometerics –nature and significance of qualitative and quantitative traits – variation – phenotypic – genotypic and environmental – heritability – genetic advance.

Selection breeding – introduction and acclimatization – self pollinated crops – genetic makeup – mass selection – pure line concept – hybridization and selection – pedigree method – bulk method – modified bulk method –mass pedigree method – single seed descent (SSD) and back cross method.

Selection breeding – cross pollinated crops – genetic makeup – mating systems – synthetics – composites – mass selection – progeny selection – recurrent selection.

Population genetics – Hardy Weinberg law – gene frequencies – random mating population - breeding asexually propagated crops – genetic makeup – clonal selection – hybridization – hybrid breeding.

Mutation breeding – types – genetic basis – mutagen and mutagenesis – handling of mutagenised population, application of mutation.

Comprehensive Plant Breeding strategy – Innovative breeding.

Special selection methods – markers – phenotypic markers – molecular markers – marker assisted selection.

PRACTICAL

Observation on reproductive and pollination systems in plants – Alternation of generation and life cycle – Description and drawing of different pollination systems – Mechanisms enforcing self and cross pollination – Morphology of pollen grains – Assessment of pollen fertility and sterility in A, B, and R line – Maintenance of A, B lines. Emasculation technique – Selfing and crossing techniques – Breeder kit – Layout of different trails – Irradiation – dosimetry – Half life period – Procedure for irradiation – Chemical mutagenesis – Molar solution – Procedure for treatment – Calculation of heterosis, PCV, GCV, heritability, genetic advance – genetic divergence – Records maintained – Wild species maintenance and utilization - screening method for specific traits – marker assisted selection.

LECTURE SCHEDULE

1. Centers of Origin – contribution of Vaviolov, Harlan and Zhukovosky – law of homologous series.
2. Conservation, evaluation and utilization - gene sanctuaries - exploration - national and international agencies engaged in conservation.
3. Incompatibility - causes of incompatibility - gametophytic and sporophytic incompatibility - significance of self - incompatibility.
4. Sterility - male sterility - genic, cytoplasmic, cytoplasmic genic male sterility and environmentally influenced male sterility - line breeding.
5. Apomixis - classification of apomixis with examples and their significance.

6. Basic biometrics –nature and significance of qualitative and quantitative traits – variation – phenotypic – genotypic and environmental – heritability – genetic advance.
7. Plant introduction - primary and secondary introduction - objectives - Acclimatization, adaptation, merits and demerits of introduction – Vilmorin principles of progeny selection - Johansen's pure line theory - Genetic structure of self-pollinated crop - Breeding methods for self pollinated crops - pure line selection - merits and demerits.
8. Mass selection and its merits and demerits - hybridization, its objectives and - types - intervarietal, interspecific - intergeneric, wide and introgressive hybridization, - choice of parents - procedures of hybridization.
9. MID SEMESTER EXAMINATION.
10. Pedigree and bulk method of selection - merits and demerits. Modified bulk method - mass pedigree method and SSD - Advantage over pedigree and bulk methods.
11. Back cross method - prerequisites and its application in transferring resistant genes - merits and demerits - Multilines, multiblends and population improvement approach in self - pollinated crops.
12. Genetic structures of a population in cross-pollinated crop Hardy - Weinberg law - Breeding methods followed in cross pollinated crops. Selection - Mass selection - Modified mass selection. Detasseling - panmixis - unit selection. Progeny testing and selection- Half sib family selection - Ear to row method, full sib family selection. Inbred or selfed family selection - Recurrent selection.
13. Heterosis, hybrid vigor and inbreeding depression. Hybrid seed production - manual emasculation and pollination - utilization of male sterility and self incompatibility.
14. Multiple Cross hybrids - advantages and disadvantages over other hybrids - Recurrent selection, types of recurrent selection and their relative advantages - synthetics and composites.
15. Genetic structure of a population of asexually reproducing crops - Clone and clonal selection - Hybridization followed by clonal selection - exploitation of heterosis to produced synthetics through poly cross technique.
16. Mutation - types of mutation - characteristics of mutation - Mutagens - Application of mutation breeding.
17. Special selection methods – markers – phenotypic markers – molecular markers – marker assisted selection.

PRACTICAL SCHEDULE

1. Pollination and Reproduction in plants - Alternation of generation and life cycle.
2. Description and drawing different pollination systems - Mechanisms enforcing self and cross pollination in crops.
3. Pollen morphology - Exine structure of different crops. Fertility and sterility in A, B, R and TGMS lines.
4. Breeder kit and its components - uses.
5. Selfing and crossing techniques in different crops.
6. Emasculation, kinds of emasculation and pollination technique.
7. Layout of different yield trials - Observing the experimental plots.
8. Irradiation - dosimetry - half life period - procedure for irradiation. Chemical mutagenesis - molar solution preparation - procedure for chemical mutagenesis.
9. Calculation of PCV, GCV, heritability, genetic advance.

10. Genetic divergence studies.
11. Maintenance of A, B and R line and TGMS lines. Hybrid seed production techniques.
12. Studies on segregating generations and maintenance of records.
13. Studies on different wild species in crop plants and wide hybridization.
14. Estimation of heterosis.
15. Screening methods – laboratory – field – for specific traits.
16. Procedure for marker assisted selection.
17. PRACTICAL EXAMINATION.

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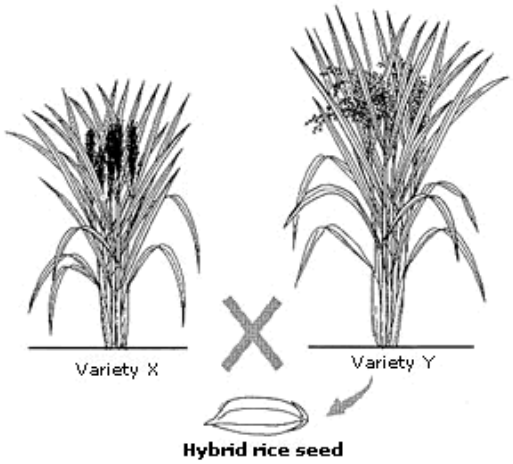
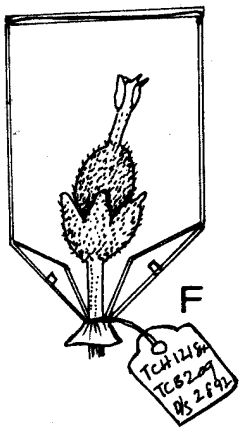
METHODS OF PLANT BREEDING

PBG 202 - PRACTICAL MANUAL





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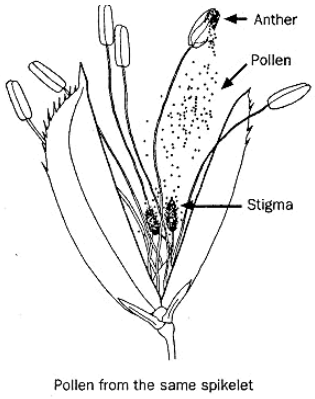

Pollen from the same spikelet

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PBG 202: METHODS OF PLANT BREEDING (1+1)
PRACTICAL MANUAL

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INDEX

Name: **I.D. No.:**

1. Pollination and Reproduction in plants - Alternation of generation and life cycle.

In the sexually reproducing crop plants definite male and female sexual organs namely androecium and gynoecium produce male gametes and female gametes respectively. The male gametes are formed from a haploid cell called microspore and the female gametes are formed from a haploid cell, megaspore.

Sporogenesis

Production of microspores and megaspores is known as sporogenesis. In anthers, microspores are formed through microsporogensis and in ovules, the megaspores are formed through megasporogenesis.

Microsporogenesis

The sporophytic cells in the pollen sacs of anther which undergo meiotic division to form haploid i.e., microspores are called microspore (MMC) or pollen mother cell (PMC) and the process is called microsporogenesis. Each PMC produce four microspores and each microspore after thickening of the wall transforms into pollen grain.

Megasporogenesis

A single sporophytic cell inside the ovule, which undergo meiotic division to form haploid megaspore, is called megaspore mother cell (MMC) and the process is called megasporogenesis. Each MMC produces four megaspores out of which three degenerate resulting in a single functional megaspore.

Gametogenesis

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

Microgametogenesis

This is nothing but the production of male gametes or sperm. On maturation of the pollen, the microspore nucleus divides mitotically to produce a generative and a vegetative or tube nucleus. The pollen is generally released in this binucleate stage. The reach of pollen over the stigma is called pollination. After the pollination, the pollen germinates. The pollen tube enters the stigma and travels down the style. The generative nucleus at this phase undergoes another mitotic division to produce two male gametes or sperm nuclei. The pollen along with the pollen tube possessing a pair of sperm nuclei is called microgametophyte. The pollen tube enters the embryo sac through micropyle and discharges the two sperm nuclei.

Megagametogenesis

The nucleus of the functional megaspore undergoes three mitotic divisions to produce eight or more nuclei. The exact number of nuclei and their arrangement varies from one species to another. The megaspore nucleus divides thrice to produce eight nuclei. Three of these nuclei move to one pole and produce a central egg cell and two synergid cells on either side. Another three nuclei migrate to the opposite pole to develop into three antipodal cells.

The two nuclei remaining in the center, the polar nuclei, fuse to form the secondary nucleus. The megaspore thus develops into a mature female gametophyte called megagametophyte or embryo sac. The development of embryo sac from a megaspore is known as megagametogeneis. The embryo sac generally contains one egg cell, two synergids with the apparent function of guiding the sperm nucleus towards the egg cell and three antipodals which forms the prothalamus cells and one diploid secondary nucleus.

Fertilization:

The fusion of one of the two sperms with the egg cell producing a diploid zygote is known as fertilization. The fusion of the remaining sperm with the secondary nucleus leading to the formation of a triploid primary endosperm nucleus is termed as **triple fusion**. The primary endosperm nucleus after several mitotic divisions develops into mature **endosperm**, which nourishes the developing embryo.

MODE OF REPRODUCTION

Knowledge of the mode of reproduction and pollination is essential for a plant breeder, because these aspects help in deciding the breeding procedures to be used for the genetic improvement of a crop species. Choice of breeding procedure depends on the mode of reproduction and pollination of a crop species.

Reproduction refers to the process by which living organisms give rise to the offspring of similar kind (species). In crop plants, the mode of reproduction is of two types: viz. 1) asexual reproduction and 2) sexual reproduction.

Asexual reproduction

Multiplication of plants without the fusion of male and female gametes is known as asexual reproduction. Asexual reproduction can occur either by vegetative plant parts or by vegetative embryos which develop without sexual fusion (apomixis). Thus asexual reproduction is of two types: viz. a) vegetative reproduction and b) apomixis.

Vegetative reproduction refers to multiplication of plants by means of various vegetative plant parts. Vegetative reproduction is again of two types: viz. i) natural vegetative reproduction and ii) artificial vegetative reproduction.

Natural vegetative reproduction

In nature, multiplication of certain plants occurs by underground stems, sub aerial stems, roots and bulbils. In some crop species, underground stems (a modified group of stems) give rise to new plants. Underground stems are of four types: viz. rhizome, tuber, corm and bulb. The examples of plants which reproduce by means of underground stems are given below:

Rhizome: Turmeric (*Curcuma domestica),* Ginger (*Zingiber officinale)*
Tuber: Potato *(Solanum tuberosum)*
Corm: Arvi *(Colocasia esculenta*), Bunda (*C. antiquorum*)
Bulb: Garlic (*Allium sativum),* onion (*A. cepa)*

Sub aerial stems include runner, sucker, stolon, etc. These stems lead to vegetative reproduction in mint (Mentha sp) rose, strawberry, banana, etc. **Bulbils** are modified forms of flower. They develop into plants when fall on the ground. Bulbils are found in garlic.

Artificial vegetative reproduction

Multiplication of plants by vegetative parts through artificial method is known as artificial vegetative reproduction. Such reproduction occurs by cuttings of stem and roots, and by layering, grafting and gootee. Examples of such reproduction are given below:

Stem cuttings: Sugarcane (Saccharum sp.) grapes (*Vitis vinifera*), roses, etc.
Root cuttings: Sweet potato, citrus, lemon, etc.
Layering, grafting and gootee are used in fruit and ornamental crops.

Apomixis

Apomixis refers to the development of seed without sexual fusion (fertilization). In apomixis embryo develops without fertilization. Thus apomixis is an asexual means of reproduction. Apomixis is found in many crop species. Reproduction in some species occurs only by apomixis. This apomixis is termed as **obligate apomixis**. But in some species sexual reproduction also occurs in addition to apomixis. Such apomixis is known as **facultative apomixis**. There are four types of apomixis: viz.
1) parthenogenesis, 2) apogamy, 3) apospory and 4) adventive embryony.

1. Parthenogenesis. Parthenogenesis refers to development of embryo from the egg cell without fertilization.

2. Apogamy. The origin of embryo from either synergids or antipodal cells of the embryosac is called as apogamy.

3. Apospory. In apospory, first diploid cell of ovule lying outside the embryosac develops into another embryosac without reduction. The embryo then develops directly from the diploid egg cell without fertilization.

4. Adventive embryony. The development of embryo directly from the diploid cells of ovule lying outside the embryosac belonging to either nucellus or integuments is referred to as adventive embryony.

Sexual reproduction

Multiplication of plants through embryos which have developed by fusion of male and female gametes is known as sexual reproduction. All the seed propagating species belong to this group.

Exercise:

1. Draw the diagrams of male and female gametophyte.
2. Differentiate between sporogenesis and gametogenesis
3. Write the ploidy level of gametes, zygote and endosperm of diploid and tetraploid cotton.

2. Description and drawing different pollination systems - Mechanisms enforcing self and cross pollination in crops.

MODE OF POLLINATION

The process by which pollen grains are transferred from anthers to stigma is referred as pollination. Pollination is of two types: viz. 1) Autogamy or self pollination and 2) Allogamy or cross pollination.

I. Autogamy

Transfer of pollen grains from the anther to the stigma of same flower is known as autogamy or self pollination. Autogamy is the closest form of inbreeding. Autogamy leads to **homozygosity**. Such species develop homozygous balance and do not exhibit significant inbreeding depression.

Mechanism promoting self-pollination

1. Bisexuality. Presence of male and female organs in the same flower is known as bisexuality. The presence of bisexual flowers is a must for self pollination. All the self pollinated plants have **hermaphrodite** flowers.

2. Homogamy. Maturation of anthers and stigma of a flower at the same time is called homogamy. As a rule, homogamy is essential for self-pollination.

3. Cleistogamy. When pollination and fertilization occur in unopened flower bud, it is known as cleistogamy. It ensures self pollination and prevents cross pollination. Cleistogamy has been reported in some varieties of wheat, barley, oats and several other grass species.

4. Chasmogamy. Opening of flowers only after the completion of pollination is known as chasmogamy. This also promotes self pollination and is found in crops like wheat, barley, rice and oats.

5. Position of Anthers. In some species, stigmas are surrounded by anthers in such a way that self pollination is ensured. Such situation is found in tomato and brinjal. In some legumes, the stamens and stigma are enclosed by the petals in such a way that self pollination is ensured. Examples are greengram, blackgram, soybean, chickpea and pea.

II. Allogamy

Transfer of pollen grains from the anther of one plant to the stigma of another plant is called allogamy or cross pollination. This is the common form of out-breeding.

Allogamy leads to **heterozygosity.** Such species develop heterozygous balance and exhibit significant inbreeding depression on selfing.

Mechanism promoting cross-pollination

1. Dicliny. It refers to unisexual flowers. This is of two types: viz. i) monoecy and ii) dioecy. When male and female flowers are separate but present in the same plants, it is known as **monoecy**. In some crops, the male and female flowers are present in the same inflorescence such as in mango, castor and banana. In some cases, they are on separate inflorescence as in maize. Other examples are cucurbits, grapes, strawberry, cassava and rubber. When staminate and pistillate flowers are present on different plants, it is called **dioecy**. It includes papaya, date palm, spinach, hemp and asparagus.

2. Dichogamy. It refers to maturation of anthers and stigma of the same flowers at different times. Dichogamy promotes cross pollination even in the hermaphrodite species. Dichogamy is of two types: viz. i) protogyny and ii) protandry. When pistil matures before anthers, it is called **protogyny** such as in pearl millet. When anthers mature before pistil, it is known as **protandry**. It is found in maize, sugarbeet and several other species.

3. Heterostyly. When styles and filaments in a flower are of different lengths, it is called heterostyly. It promotes cross pollination, such as linseed.

4. Herkogamy. Hinderance to self-pollination due to some physical barriers such as presence of hyline membrane around the anther is known as herkogamy. Such membrane does not allow the dehiscence of pollen and prevents self-pollination such as in alfalfa.

5. Self incompatibility: The inability of fertile pollens to fertilize the same flower is referred to as self incompatibility. It prevents self-pollination and promotes cross pollination. Self incompatibility is found in several crop species like *Brassica, Radish, Nicotiana,* and many grass species. It is of two types **sporophytic** and **gametophytic**.

6. Male sterility. In some species, the pollen grains are non functional. Such condition is known as male sterility. It prevents self-pollination and promotes cross pollination. It is of three types: viz. **genetic, cytoplasmic** and **cytoplasmic** genetic. It is a useful tool in hybrid seed production.

Study of **floral biology** and aforesaid mechanisms is essential for determining the mode of pollination of various crop species. Moreover, if selfing has adverse effects on seed setting and general vigour, it indicates that the species is cross pollinated. If selfing does not have any adverse effect on these characters, it suggests that the species is self-pollinated.

The percentage of cross pollination can be determined by growing a seed mixture of two different varieties together. The two varieties should have marker characters say green and pigmented plants. The seeds are harvested from the recessive (green) variety

and grown next year in separate field. The proportion of pigmented plants in green variety will indicate the percentage of **outcrossing** or cross pollination.

Significance of pollination

The mode of pollination plays an important role in plant breeding. It has impact on five important aspects : viz. 1) gene action, 2) genetic constitution, 3) adaptability, 4) genetic purity and 5) transfer of genes.

Summary of modes of reproduction found in crop plants

Terms	*Brief description/ definition*
A. Asexual Reproduction	Multiplication of plants bypassing sexual process
1. Vegetative Reproduction	Multiplication by vegetative plant parts.
a. Natural	Multiplication by Rhizome, tuber, corm, bulb, runner, suckers and stolon.
b. Artificial	Multiplication by stem and root cuttings, grafting, layering and budding.
2. Apomixis	Development of embryo without sexual fusion.
Parthogenesis	Development of embryo from egg cell without fertilization.
Apogamy	Development of embryo either from synergids or antipodal cells.
Apospory	Origin of embryo from diploid egg cell of another embryosac developed from diploid tissues.
Adventive Embryony	Origin of embryosac directly from diploid cells belonging to either nucellus or integument.
B. Sexual Reproduction	Multiplication of plants by fertilized embryos.
Autogamy	Fertilization of ovules by the pollens of same flower.
Allogamy	Fertilization of ovules by the pollens of another plant.

Classification of crop plants based on mode of pollination and mode of reproduction

Mode of pollination and reproduction	Examples of crop plants
A. Autogamous Species	
1. Seed Propagated	Rice, Wheat, Barley, Oats, Chickpea, Pea, Cowpea, Lentil, Green gram, Black gram, Soybean, Common bean, Moth bean, Linseed, Sesame, Khesari, Sunhemp, Chillies, Brinjal, Tomato, Okra, Peanut, etc.
2. Vegetatively Propagated	Potato
B. Allogamous Species	
1. Seed Propagated	Corn, Pearlmillet, Rye, Alfalfa, Radish, Cabbage, Sunflower, Sugarbeet, Castor, Red clover, White clover, Safflower, Spinach, Onion, Garlic, Turnip, Squash, Muskmelon, Watermelon, Cucumber, Pumpkin, Kenaf, Oilpalm, Carrot, Coconut, Papaya, etc.
2. Vegetatively propagated	Sugarcane, Coffee, Cocoa, Tea, Apple, Pears, Peaches, Cherries, grapes, Almond Strawberries, Pine apple, Banana, Cashew, Irish, Cassava, Taro, Rubber, etc.
C. Often Allogamous Species (seed propagated)	Sorghum, Cotton, Triticale, Rai, Pigeonpea, Tobacco.

Exercise

1. Draw the diagrams of crop adaptation to self-pollination and cross pollination
2. How will you assess the extent of cross-pollination in a plant –species?
3. Differentiate between the following
    a) Autogamy and allogamy
    b) Apogamy and apospory
    c) Cleistogamy and chasmogamy

3. Pollen morphology - Fertility and sterility in A, B, R and TGMS lines.

1. Palynology

This is the science involving the study of pollens. The pollen has a very minute structure. It is unicellular and usually round although it may be oval, pyramidal, polyhedral etc. It is provided with two coats-an inner, delicate, cellulose layer called **intine** and an outer tough, cutinised layer called exine or **extine**. The exine is often sculptured or provided with spines, warts etc., occasionally, it is smooth. The exine may have a waxy coating to render the pollen more or less waterproof. Very often, there are some definitely thinner circular spots or slits in the exine called **germ pores** or **slits**. These weak spots are utilized during the germination of the pollen.

2. Preparation of Acetocarmine Stain ($C_{22}H_2O_{13}$)

It is one of the most widely used stain for pollen study. A mixture of 4 ml glacial acetic acid and 55 ml of distilled water is boiled. A quantity of 1 g of carmine (according to the strength required) is added to 100 ml of the above mixture at about boiling point and then boiled for few minutes. After boiling, the contents are removed from the flame and allowed to cool and filtered in a clean bottle. The filtrate is reddish in colour and known as 1% acetocarmine. Ferric chloride or ferric acetate may be added if necessary for deep staining and preservation.

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

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

Types of male sterility, maintenance and uses:

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

a) Cytoplasmic male sterility (CMS)
b) Genetic male sterility (GMS)
c) Cytoplasmic-genetic male sterility (CGMS)
d) Environmental induced male sterility which is again sub divided in to
    i) TGMS (Theromosensitive)
    ii) PGMS (Photo sensitive)

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

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

R line or restorer line: It is other wise known as Restorer line which restores fertility in the A line. The crossing between A x R lines results in F_1 fertile hybrid seeds which is of commercial value.

Pollen fertility count

a. Different crop species

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

b) A, B & R Lines of rice, cumbu.

Lines	Number of pollen grains		Total	Percentage of pollen fertility
	Unstained	Stained		
A				
B				
R				

Exercise:

1. Observe and record pollen morphology of the given crop species.
2. What type of male sterility system is commercially being exploited in the crops like Rice, Sorghum, Cumbu and Redgram?
3. Differentiate TGMS and PGMS.
4. Distinguish between ::
   Genetic and cytoplasmic genetic male sterility.

4. Breeder's kit and its components – uses.

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

S.No.	Items	Purpose
1.	Magnifying lens	To observe small flowers, stigmatic surface, dehiscence of anthers etc.
2.	Forceps	Fine forceps are required for emasculation
3.	Scissors	Required to remove unwanted buds, awns, etc.,
4.	Needles	Required to open small buds and separating the floral parts.
5.	Brushes	Camel hair brushes of size 3 or 4 for collection of pollen and transfer to stigma.
6.	Bags	Parchment paper bag, khaki cloth bags, muslin cloth bag, and paper bags of different sizes for different crops.
7.	Alcohol or Methylated spirit	A small vial of alcohol or methylated spirit is required to sterilize forceps, scissors, needles, brushes etc.,
8.	Tags	Paper, cardboard or aluminium tags are required for labeling the units in the field. In the case of paper or cardboard tags, they have to be dipped in wax after labeling and tags are tied in bamboo stakes.
9.	Meter scale	Required for plant measurement in the field.
10.	Field note books	Field note books are required to note down daily observation in the field, regarding germination, flowering, morphological description, initial and final stand, wet weight of grains, haulms etc.

Exercise

1. Draw the different components of Breeder's kit.

5. Selfing and crossing techniques in different crops

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

Selfing

In the selfing of cross-pollinated species, it is essential that the flower are bagged or otherwise protected to prevent natural cross-pollination. Selfing and crossing are essential in crop breeding. It is important that the breeder, master these techniques in order to manipulate the pollination according to his needs. The exact procedure that he may use to ensure self or cross pollination of specific plants will depend on the particular species with which he is working. The structure of the flowers in the species determine manner of pollination. For these reasons, the breeder should acquaint himself with the **flowering habit** of the crop.

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

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

Emasculation

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

Exercise

1 Effecting self-pollination in a cross pollinated crop (or) effecting cross pollination in a self-pollinated crop which one is easier – why?

2 Note down the type and size of bags used in crossing blocks of cumbu, maize cholam, cotton, sunflower and castor and offer your remarks.

6. Emasculation, kinds of emasculation and pollination techniques

Methods of Emasculation

1. Hand Emasculation

In species with large flowers, removal of anthers is possible with the help of forceps. It is done before anther dehiscence. It is generally done between 4 and 6 PM one day before anthers dehisce. It is always desirable to remove other young flowers located close to the emasculated flower to avoid confusion. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed with the help of forceps. Sometimes corolla may be totally removed along with **epipetalous stamens** e.g. gingelly.

In cereals, one third of the empty glumes will be clipped off with scissors to expose anthers. In wheat and oats, the florets are retained after removing the anthers without damaging the spikelets. In all cases, gynoecium should not be injured. An efficient emasculation technique should prevent self pollination and produce high percentage of seed set on cross pollination.

2. Suction Method

It is useful in species with small flowers. Emasculation is done in the morning immediately after the flowers open. A thin rubber or a glass tube attached to a suction hose is used to suck the anthers from the flowers. The amount of suction used is very important which should be sufficient to suck the pollen and anthers but not gynoecium. In this method considerable self-pollination, upto 10% is like to occur. Washing the stigma with a jet of water may help in reducing self-pollination, However self pollination can not be eliminated in this method.

3. Hot Water Treatment

Pollen grains are more sensitive than female reproductive organs to both genetic and environmental factors. In case of hot water emasculation, the temperature of water and duration of treatment vary from crop to crop. It is determined for every species. For sorghum 42-48^{O}C for 10 minutes is found to be suitable. In the case of rice, 10 minutes treatments with 40-44^{O}C is adequate. Treatment is given before the anthers dehiscence and prior to the opening of the flower. Hot water is generally carried in thermos flask and whole inflorescence is immersed in hot water.

4. Alcohol Treatment

It is not commonly used. The method consists of immersing the inflorescence in alcohol of suitable concentration for a brief period followed by rinsing with water. In

Lucerne the inflorescence immersed in 57% alcohol for10 second was highly effective. It is better method of emasculation than suction method.

5. Cold Treatment

Cold treatment like hot water treatment kills the pollen grains without damaging gynoecium. In the case of rice, treatment with cold water $0.6^{O}C$ kills the pollen grains without affecting the gynoecium. This is less effective than hot water treatment.

6. Genetic Emasculation

Genetic/ cytoplasmic male sterility may be used to eliminate the process of emasculation. This is useful in the commercial production of hybrids in maize, sorghum pearlmillet, onion, cotton, and rice, etc.,

In many species of self-incompatible cases, also emasculation is not necessary, because self-fertilization will not take place. Protogyny will also facilitate crossing without emasculation (e.g.) Cumbu.

7. Use of Gametocide

Also known as chemical hybridizing agents (CHA) chemicals which selectively kills the male gamete without affecting the female gamete. eg. Ethrel, Sodium methyl arsenate, Zinc methyl arsenate in rice, Maleic hydrazide for cotton and wheat.

Bagging

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

Crossing

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

Tagging

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

1. Date of emasculation:
2. Date of pollination
3. Parentage:
4. No. of flowers emasculated:

Exercise:

1. Observe and illustrate different crossing techniques followed in the different crops.
2. Among the emasculation techniques, which is the most efficient one and why?

7. Lay out of different yield trials

Laying out of Field Experiments

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

Any designing of experiments involves three major steps.

1. Selection of experimental units

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

2. Fixing of treatments

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

3. Arrangement of treatments in the experimental Units.

It comprises of three basic principles of design

a) **Replication:** repetition of treatments
b) **Randomization:** unbiased allocation of treatments to the experimental units.
c) **Local control:** minimizing the effect of heterogeneity of the experimental units.

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

Types of basic experimental designs

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

Among these, RBD is the widely used design.

Laying Out of RBD

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

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

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

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

D. Computation of Critical Difference (CD)

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

RT : Row trial

Row trial is generally conducted in F_3 and F_4, when the seeds are not sufficient for replication with individual plant progeny rows. Each row consists of about 20 or more plants. Individual plants with desirable characteristics are selected from superior progeny rows. Pest, Disease and lodging susceptible progenies with undesirable characteristics are eliminated.

RRT – Replicated Row Trial

It is generally conducted from F_3 generation onwards. Depending on availability of seeds, 3-4 more rows are grown for each progeny to facilitate comparison among progenies adopting suitable replications. Families, which have become reasonably homozygous may be harvested in bulk. From those families showing segregation, single plants are selected for characters under study. The breeder has to visually assess the yielding potential of progenies and reject the inferior ones in the field and the yield potential has to be assessed in the laboratory for confirmation.

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

It is conducted from F_5 generation onwards. Preliminary yield trial with three or more replications are conduct to evaluated the comparative performance of the culture and to identity the superior cultures among them. The cultures are evaluated for plant height, lodging, pest and disease resistance, flowering time, duration and yield, etc., Quality tests may also be carried out. Standard commercial varieties must be included as checks for comparison. Ten to fifteen outstanding cultures, if superior to checks, would be advanced to the Advanced yield trails.

AYT – Advanced Yield Trial

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

Multi location trial is conducted from F_{13} onwards for 3 years by the Research Station Scientists. Multilocation Trial are useful for suitability studies i.e. whether a particular culture is able to perform well in all the locations or not. Stable performance of a culture over all the locations will be promoted to ART.

ART – Adaptive Research Trial

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

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

Exercise:

1. Visit the millet breeding station and observe the different field experiments laid out. Offer your comments.

8. Physical and Chemical mutagenesis.

Physical Mutagens

Physical mutagens include various types of radiation, viz X-rays, gamma rays, alpha particles, beta particles, fast and thermal (slow) neutrons and ultra violet rays. A brief description of these mutagens is presented below:

Commonly used physical mutagens (radiations), their properties and mode of action.

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

Note: particulate refers to particle emitting property DI = Densely ionizing, SI = Sparasely ionizing.

1. X-rays

X-rays were first discovered by Roentgen in 1895. the wavelengths of X-rays vary from 10^{-11} to 10^{-7}. They are sparsely ionizing and highly penetrating. They are generated in X-rays machines. X-rays can break chromosomes and produce all types of mutations in nucleotides, viz. addition, deletion, inversion, transposition, transitions and transversions. These changes are brought out by adding oxygen to deoxyribose, removing amino or hydroxyl group and forming peroxides. X-rays were first used by Muller in 1927 for induction of mutations in Drosophila. In plants, Stadler in 1928 first used X-rays for induction of mutations in barley. Now X-rays are commonly used for induction of mutations in various crop plants, X-rays induce mutations by forming free radicals and ions.

2. Gamma rays

Gamma rays are identical to X-rays in most of the physical properties and biological effects. But gamma rays have shorter wave length than X-rays and are more penetrating than gamma rays. They are generated from radioactive decay of some elements like 14C, 60C, radium etc. Of these, cobalt 60 is commonly used for the production of Gamma rays. Gamma rays cause chromosomal and gene mutations like X-rays by ejecting electrons from the atoms of tissues through which they pass. Now a days, gamma rays are also widely used for induction of mutations in various crop plants.

Gamma chamber

It is a compact, self contained irradiation unit offering an irradiation volume of approximately 1000CC with adequate protection and sealed so that, the radiation leakage outside the unit is well below the maximum permissible dose level. No extra shield is required in the laboratory. The chamber at TNAU, Coimbatore was installed during October 1972. The main unit essentially consists of three parts.

a) Source cage, b) Biological shield for source, c) central shaft with irradiation chamber.

a) Source cage: holds the irradiation source in an angular cylinder, the co-axial hole in the center of the cage provides space for irradiation chamber. The cage is designed to hold 18 to 20 pencils containing cobalt 60, in the form of pellets. The design of the source cage optimize to provide maximum uniformity of dose rate in the site of irradiation chamber.

b) Biological shield: It consists of two parts I) Main outer shield ii) Removable plug. The radiation source is housed in the main outer shield which is designed to reduce the radiation on the outer surface or to a value less than maximum permissible limit for laboratories. The inner removable plug is a cylindrical lead plug with a co-axial hole and act as a guide for the shaft. The removable plug permits easy loading and unloading of radiation source in the main field.

c) Central shaft with irradiation chamber: Central shaft is a solid lead cylinder at the center of which, sample chamber is located. The size of the sample chamber is 14.3cm high with 10 cm diameter. The purpose of central lead is to provide shielding during upward and downward movement of the sample chamber.

Sample chamber and control plan

The sample chamber is raised or lowered by a wire rope, using system of pulling by a rotating drum. The drum is rotated by an electric motor or self locking rotating gear. The circuit which controls the movement includes a synchronised motor, operator, timer and upward and downward switches. Provision is made for manual as well as automatic operation and the mode of operation is decided by panel switch.

Instructions for Operation

a) Switch on the power key. Push the 'up'. The chamber will come up.
b) Open the chamber and place the materials to be irradiated inside and close the chamber.
c) Set the irradiation time on the timer
d) Put the timer switch on auto. Push the 'down' switch, at the end of the prescribed time, the sample automatically comes out. If the irradiation is to be terminated, prior to the prescribed time, put the switch in the manual operation and pres the 'up' switch.

Procedure for chemical mutagenesis

The chemical mutagens can be divided into four groups, viz. 1) alkylating agents, 2) base analogues, 3) acridine dyes, and 4) others. A brief description of some commonly used chemicals of these groups is presented below.

Some commonly used chemical mutagens and their mode of action

Group of mutagen	*Name of chemical*	*Mode of action*
1. Alkylating Agents	Ethyl methane Sulphonate Methyl Methane Sulphonate Ethyl Ethane Sulphonate Ethylene Imines	AT ⟷ GC Transitions Transitions GC ⟷ AT Transitions Transitions
2. Base Analogues	5 Bromo Uracil 2 Amino purine	AT ⟷ GC Transitions AT ⟷ GC Transitions
3. Acridine Dyes	Acriflavin, Proflavin	Deletion, addition and frame shifts.
4. Others	Nitrous Acid Hydroxylamine Sodium Azide	AT ⟷ GC Transitions GC ⟷ AT Transitions Transitions

The speed of hydrolysis of the chemical mutagens is usually measured by the half life of the chemicals. Half life is the time required for disappearance of the half of the initial amount of active reaction agent. The following table gives the half life in hours at different temperatures.

Chemicals	Temperature		
	$20^{O}C$	$30^{O}C$	$37^{O}C$
MMS (hours)	68	20	9.1
EMS	93	26	10.4
DES	3.3	1	-
NMU	-	35	-
NEU	-	84	-

In the case of DES the mutagenic solution should be changed at every half an hour to get good results. Half life is the function of temperature and pH for a particular compound.

One should be extremely careful in handling alkylating agents since most of them are carcinogenic. Especially for ethylene imine, it should be handled under aerated conditions. EMS though not dangerous, it should not be pipetted out by mouth. Besides the alkylating agents, we are also having chemical mutagens like, Base analogues, Acridine dyes, Antibiotics and other miscellaneous chemicals.

Treatment of seeds with mutagenic chemicals.

Materials required:- conical flask, beaker, pipette, glass rods, measuring cylinder, stop watch, distilled water and phosphate buffer.

Method: - Mutagenic chemical is diluted to the required concentration by using distilled water. To prepare the molar concentration of DES, the method is

$$\frac{\text{Molecular weight}}{\text{Specific gravity}} \times \text{a.i. (purity percentage) (active ingredient)}$$

$$\text{Eg. DES} = \frac{154}{1.18} \times \frac{100}{99} = 131 \text{ CC.}$$

131 CC dissolved in one litre will give 1 molar solution.

Seeds have to be soaked in the distilled water for different hours depending upon the seeds, to initiate biochemical reactions. The chemical action is found to be affected by the frequency and spectrum of mutagen depending upon the stage of cell division, during the process of germination. If the chemical treatment is synchronized with DNA synthesis stage (G_1, S and G_2) then we can get better results.

The presoaked seeds are taken in a flask and chemical is added. Usually the quantity of the chemical is ten times the volume of seeds. Intermittent shaking should be given to ensure uniform exposure of the chemicals. The chemical should be drained after the treatment time is over. The seeds should be washed thoroughly in running tap water, immediately for not less than 30 minutes. After washing, the seeds should be dried in between the filter paper folds. Seeds are to be arranged in germination tray with equal spacing. Trays are kept in a controlled environment of temperature and humity. Periodical observation on germination upto 10-15 days is needed. From the germination percentage, we can assess the LD50 dose.

Exercise

1. Define: Mutation
2. Write the formulae for mutagenic effectiveness and mutagenic efficiency .
3. Mutations are called as single stoke event why?
4. Visit the gamma ray chamber and treat the given samples for different doses of gamma rays.
5. Find out the LD 50 value for the given specimen with respect to germination %, shoot and root length?

9. Calculation of PCV, GCV, heritability and genetic advance

The phenotype may be described according to a mathematical model to facilitate statistical analysis and interpretation. The phenotypic mean i.e. x of a given genotype from the trial may be expressed as m,

$\overline{x} = \mu + g + e + ge$ where,

x = phenotypic mean

μ = General population mean

g = effect of genotype

e = effect of environment

ge = interaction between genotype and environment

Analysis of variance for genotypes grown in a replicated trial according to RBD

Source of variation	d.f.	Expectation of MS
Replications	r-1	$\sigma_e^2 + g\sigma_r^2$
Genotypes	g-1	$\sigma_e^2 + r\sigma_g^2$
Error	(r-1) (g-1)	σ_e^2
Total	(rg)-1	

g and r are the number of genotypes and replications respectively; and σ_e^2, σ_r^2 and σ_g^2 denote the variances due to error, replications and genotypes respectively.

Genotypic variance (σ_g^2)=(MS due to genotypes – MS due to error)/R

Phenotypic variance (σ_p^2) = $\sigma_g^2 + \sigma_e^2$

Genotypic Co-efficient of variation

$$GCV := \frac{\sigma_g^2}{\mu} \times 100$$

Phenotypic Co-efficient of variation

$$PCV = \frac{\sigma_p^2}{\mu} \times 100$$

Heritability

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Genetic Advance

$$GA = \frac{\sigma_g^2}{\sqrt{\sigma_p^2}} \times K$$ where,

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

Problem:

In redgram, 20 genotypes were evaluatejd for pods per plant. The genotypes were raised in RBD with three replications. Estimate the different variability parameters from the given data and interpret the results.

Genotypes	RI	RII	RIII
L_1 x T_1	210.8	177.2	190.7
L_1 x T_2	240.2	159.0	147.4
L_1 x T_3	206.9	140.2	169.7
L_1 x T_4	126.7	87.3	71.5
L_1 x T_5	139.8	112.5	87.9
L_2 x T_1	135.5	119.1	104.8
L_2 x T_2	174.3	121.3	107.9
L_2 x T_3	145.7	103.4	118.1
L_2 x T_4	86.5	71.3	91.6
L_2 x T_5	147.4	112.2	72.4
L_3 x T_1	135.7	115.7	104.1
L_3 x T_2	186.7	108.2	117.4
L_3 x T_3	106.2	85.3	66.7
L_3 x T_4	99.8	98.4	70.6
L_3 x T_5	141.3	115.3	108.3
L_4 x T_1	115.7	93.4	70.6
L_4 x T_2	228.6	142.3	159.4
L_4 x T_3	99.5	101.7	69.8
L_4 x T_4	97.8	78.1	61.6
L_4 x T_5	103.4	88.4	63.5

10. Genetic divergence studies

The sum total of hereditary material i.e. all the alleles of various genes, present in a crop species and its wild relatives is referred to as germplasm. This is also known as genetic resources or gene pool or genetic stock. Important features of plant genetic resources are given below.

1. Genetic 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 both cultivated and wild species and relatives of crop plants.
4. Germplasm is collected from centres of diversity, gene banks, gene sanctuaries, farmer's fields, markers and seed companies.
5. Germplams 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 Conservation

Conservation refers to protection of genetic diversity of crop plants from genetic erosion. There are two important methods of germpalsm conservation or preservation. i) In-situ conservation and ex situ conservation. These are described below.

i) In - situ conservation:

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

Merits: In this method of conservation, the wild species and the compete natural or seminatural ecosystems are preserved together.

Demerits:

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

Ex - situ conservation:

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

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

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

1) Seed banks:

Germplam is stored as seeds of various genotypes. Seed conservation is quite easy, relatively safe and needs minimum space .

Seeds are classified, on the basis of their storability into two major groups. 1) Orthodox 2) Recalcitrant.

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

Recalcitrant: Seeds which show very drastic loss in viability with a decrease in moisture content below 12 to 13% are known as recalcitrant seeds. (e.g) citrus, cocoa, coffee, rubber, oilpalm, mango, jack fruit etc.

Seed storage:

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

Base collections: Seeds can be conserved under long term (50 to 100 years), at about $-20^{O}C$ with 5% moisture content. They are disturbed only for regeneration.

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

Working collections: Seeds are stored for 3-5 years at $5\text{-}10^{O}C$ and the usually contain about 10% moisture. Such materials are regularly used in crop improvement programmes.

2. Plant Bank : (Field or plant bank)is an orchard or a field in which accessions of fruit trees or vegetatively propagated crops are grown and maintained.

Limitations:
1. Require large areas
2. Expensive to establish and maintain.
3. Prone to damage from disease and insect attacks.
4. Man – made
5. Natural disasters
6. Human errors in handling.

3. Shoot tip banks:

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

1. Each genotype can be conserved indefinitely free from virus or other pathogens.
2. It is advantageous for vegetatively propagated crops like potato, sweet potato, cassava etc., because seed production in these crops is poor
3. Vegetatively propagated material can be saved from natural disasters or pathogen attack.
4. Long regeneration cycle can be envisaged from meristem cultures.
5. Regeneration of meristerms is extremely easy.
6. Plant species having recalcitrant seeds can be easily conserved by meristem cultures.

4. Cell and organ banks:

A germplasm collection based on cryopreserved (at $-196^{O}C$ in liquid nitrogen) embryogenic cell cultures, somatic/ zygotic embryos they be called cell and organ bank.

5. DNA banks:

In these banks, DNA segments from the genomes of germplasm accessions are maintained and conserved.

Germplam evaluation

Evaluation refers to screening of gemplasms in respect of morphological, genetical, economic, biochemical, physiological, pathological and entomological attributes. Evaluation of germplasm is essential from following angles.

1. To identify gene sources for resistance to biotic and abiotic stresses, earliness, dwarfness, productivity and quality characters.
2. To classify the germplasm into various groups
3. To get a clear pictures about the significance of individual germplasm line.

IPGRI, Rome has developed model list of descriptors (= characters) for which germplasm accessions of various crops should be evaluated. The evaluation of germplasm is done in three different places viz., (1) in the field (2) in green house a) 3) in the laboratory.

Germplasm cataloguing, Data storage and Retrieval.

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

Gene banks for various crops in India.

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

List of important International Institutes

Name	Institute	Activity
IRRI	International Rice Research Institute, Los Banos, Philippines	Tropical rice Rice collection: 42,000
CIMMYT	Centre International de-Mejoramients de maize Y Trigo, El Baton, Mexico	Maize and wheat (Triticale, barely, sorghum) Maize collection – 8000
CIAT	Center International de-agricultural Tropical Palmira, Columbia	Cassava and beans, (also maize and rice) in collobaration with CIMMYT and IRRI
IITA	International Institute of Tropical Agriculture, Ibadan, Nigeria.	Grain legumes, roots, and tubers, farming systems.
CIP	Centre International de-papa-Lima. Peru	Potatoes
ICRISAT	International Crops Research Institute, for Semi-Arid Tropics, Hyderabad, India	Sorghum, Groundnut, Cumbu, Bengalgram, Redgram.
WARDA	West African Rice Development Association, Monrovia, Liberia	Regional Cooperative Rice Research in Collobaration with IITA and IRRI
IPGRI	International Plant Genetic Research Institute, Rome Italy	Genetic conservation.

Exercise:

1. Visit the cold storage unit installed at the department of cotton and what are the infrastructure facilities needed to erect a cold storage unit
2. Differentiate between the following
   a) Base, Active and Working Collections.
3. Visit to CPMB and observe the invitro germplasm.

11. Maintenance of A, B , R and TGMS lines. Hybrid seed production techniques.

I. RICE

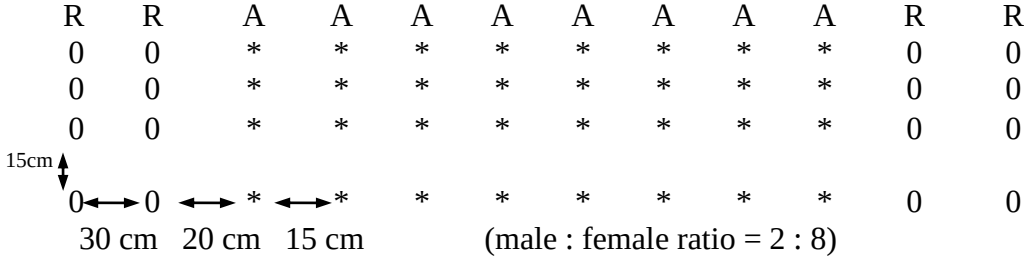
Hybrid Seed Production

Hybrid vigour in rice has been first reported by Jones (1926). This has led to speculation regarding the production of hybrid rice by utilising cytoplasmic male sterility. Most japonica rice has normal cytoplasm, but indica varieties with sterile cytoplasm and fertility restoring system have been identified. But difficulties have been encountered in obtaining sufficient seed set by cross pollination to make hybrid rice seed production economically feasible. After the implementation of UNDP/FAO project entitled "Development and use of hybrid rice technology in India" - the hybrid rice production in India has become a success story.

Hybrid rice seeds were produced using (cytoplasmic genic male sterility) three line system. The two genes Rf_1 and Rf_2 are the genes for fertility restoration.

The process of hybrid rice production involves continuous supply of agronomically improved cytoplasmic male sterile line (A), maintainer line (B) and fertility restorer (R) line in system. Maintainer and restorer lines are maintained by selfing, while CMS line and F_1 seeds are produced with efforts to enhance cross pollination in field. F and S refer to fertile and sterile cytoplasm. Rf and rf are fertility restoring and non restoring gene respectively.

Row ratio and spacing of A and R lines in the main field



Technique of hybrid rice seed production

The following points are to be taken in to account for a successful hybrid rice production.

1) **Choice of field** : Fertile soil, protected irrigation and drainage system, sufficient sunshine. No serious disease and insect problem.

2) **Isolation** : To ensure purity of hybrid seed and avoid pollination by unwanted pollen isolation is a must.

a. Space isolation: No other rice varieties should be grown except pollen parent with a range of 100m distance.

b. Time isolation: a time of over 20 days is practiced (The heading stage of other variety over a 100m range should be 20 days earlier or later over the MS line).

c. Barrier isolators : Topographic features like wood lot, tall crops to a distance of 30m/artificial obstacles of (plastic sheet) above 2m height.

3) Optimum time for heading and flowering

Favourable climatic condition for normal flowering are

(i) Mean temperature 24-28°C
(ii) Relative humidity 70-80%
(iii) Day and night temperature difference 8-10°C.
(iv) Sufficient sunshine
(v) Sufficient breeze.

4) Synchronization of flowering

As the seed set on MS line depends on cross pollination it is most important to synchronize the heading date of the male and female parents. In addition, in order to extend the pollen supply time, the male parent is usually seeded twice or thrice at an interval of 5-7 days.

5) Row ratio, row direction and planting pattern

Row ratio refers to the ratio of number of rows of the male parent to that of the female parent in the hybrid seed production field. The layout of row ratio depends on

(i) The growth duration of the R line
(ii) Growth vigor of the R line
(iii) Amount of pollen shed and
(iv) Plant height of the R line.

The principles include

* R line should have enough pollen to provide
* the row direction should be nearly perpendicular to the direction of winds prevailing at heading stage to facilitate cross pollination.

Practically, a row ratio of 2:8 is currently widely used in indica hybrid seed production.

Generally, the R line is transplanted with two to three seedlings per hill and separated by a spacing of 15cm from plant to plant, 30cm from one row of restorer to another and 20cm from CMS line. The MS line is transplanted with one to two seedlings per hill with a spacing of 15x15 cm.

A good population structure to get more seed yield is given below :

a) Seedling/hill	b) Hills/sq.m	c) effective tillers/sq.m
A line = 1-2	A line = 30	A line = 300
R line = 2-3	R line = 5	R line = 120

6) Prediction and adjustment of heading date

Even if the seeding interval between both parents is accurately determined, the synchronization of their flowering might not still be attained because of variation in temperature and difference in field management. Hence it is necessary to predict their heading date in order to take measures as early as possible to make necessary adjustments by examining the primordial initiation of panicle.

Adjustment of flowering date can be made by applying quick releasing nitrogen fertilizer on the earlier developing parent and the later developing parent should be sprayed with 2% solution DAP. By this measure a difference of 4 to 5 days may be adjusted.

7) Leaf clipping, gibberellin application and supplementary pollination

These techniques are very effective for increasing the outcrossing rate.

a) Leaf clipping : The leaves taller than the panicles are the main obstacles to cross pollination and, therefore, should be cut back. Generally leaf clipping is undertaken 1-2 days before the initial heading stage, and more than 2/3 rd of the blades of flag leaves are cut back from the top.

b) Application of gibberellin (GA_3) GA_3 can adjust physiological and biochemical metabolism of rice plant and helps in hybrid seed production by stimulating the elongation of young cells. In most of the CMS lines, about 20-30% of spikelets of a panicle are inside the flag leaf sheath (exertion is only 70%). GA_3 affects exertion of panicle completely out of flag leaf sheath. In India recommended dose of GA_3 is 50g/ha using knapsack sprayer and 25g/ha with ultra low volume sprayer.

Advantage of GA_3 application

* enhances panicle and stigma exertion
* speed up growth of late tillers and increase effective tillers
* flag leaf angle is increased
* reduces unfilled grains
* enhances seed setting and seed yield

Spraying stage : 5% of panicle emergence

Spraying time : 8-10AM is the best time.

c) Supplementary pollination : Shaking the R lines panicles by rope-pulling or rod driving during anthesis can enhance the crossing rate. This is carried out during peak anthesis (10-12 AM).

8) Rogueing

To get 98% purity of CMS lines and R lines, in addition to strict isolation, a thorough rogueing is also necessary.

9) Harvesting and processing

- the male parent harvested first
- care should be taken to avoid admixture of male and female lines.
- female line should be threshed separately in a well cleaned threshing floor
- seed field dried in shade to 12% moisture content
- packed in suitable, cleaned gunny bags after grading

Hybrid Rice CORH - 1 (MGR Rice) : Released in1994

Short duration, medium fine grain (Parentage : IR 62829A x IR10198-66-2R)

Breeding method	:	Three line Breeding
Season	:	May-June (Kar-Kuruvai)
Duration	:	110-115 days
Yield	:	6380 kg/ha
Area of adaptation	:	Coimbatore, Madurai, Chengalput, Salem, Nagapattinam, Periyar Districts.

II. PIGEONPEA

Hybrid Seed Production of (CoRH 1)

In the exploitation of hybrid vigour for commercial cultivation, efficient production of hybrid seed is essential for which a full knowledge of the various steps involved in hybrid seed production is necessary to achieve the twin objectives of maximizing the hybrid seed production and improvement in quality of hybrid seed.

For hybrid seed production, a ratio of 4:1 of male sterile pollen parent is adopted. Sufficient isolation distance i.e., more than 200 metres for the hybrid seed production plot is needed. There should not be any pigeonpea crop within a radius of 200 metres from the seed production plot. Since the male sterility is maintained in heterozygous state following the test cross principle, there would be fertile and sterile plants in the ratio 1:1 in the male sterile population. It is therefore imperative to remove the male fertile plants in the male sterile population before flower opening. The rogueing should be done thoroughly to avoid contamination by the pollen from any left out fertile plants.

Steps involved in hybrid seed production

1. Selection of site

(i) Fertile field with an irrigation source
(ii) Previous crop should not be pigeonpea
(iii) Isolation distance of 200m from any other variety of pigeonpea.

2. Fertilizer

(i) Farm yard manure @ 20 cart loads per hectare
(ii) 25 Kg N + 50 Kg of P as basal application

3. Sowing

(i) The female and male parents are sown in the ratio of 4:1 with two border rows of pollinator parent.
(ii) The pollen parent (ICPL 87109) should be sown one week after sowing the female parent (MS T.21).
(iii) Row spacing of 45 cm.
(iv) Plant to plant spacing should be 15 cm.
(v) Dibble 2-3 seeds per hill for the female parent
(vi) Seed rate (per hectare) for 4:1 ratio 40 Kg of female parent, 5 kg of male parent.
(vii) Sowing should be done during first fortnight of June or first fortnight of December.
(viii) The whole plot should be bordered with sunflower to increase the bee activity to effect cross pollination.

4. Irrigation

(i) First irrigation after sowing and a life irrigation 2-3 days after sowing.

(ii) irrigate the plot at 7-10 days interval depending upon the moisture in the field

5. Rogueing

(a) Male sterile line or female parent :

(i) Remove the off type plants

(ii) Remove the male fertile plants by examining the colour of the anthers (yellow) at the time of first flower formation, one-day before flower opening.

(iii) Rogueing should be completed in 7-10 days time

(iv) Remove the late flowering plants also.

(b) Male fertile line or pollen parent :

(i) Rogue out off types.

(ii) Remove the immature pods set in the plants from time to time to induce continuous flowering and to ensure pollen availability for a longer period.

6. Harvesting

Collect the pods from the female parent i.e., male sterile parent. This will give the hybrid seeds.

Production and maintenance of male sterile line

Genetic male sterility is utilized in hybrid seed production. In case of pigeonpea, the male sterile line will segregate in 1:1 ratio of fertile to sterile. For the maintenance of the male sterile population (to be raised under isolation), the male sterile plants have to be identified and tagged and the fertile plants have to be retained without tagging. The male sterile lines will be pollinated naturally by the pollen from the male fertile plants in the population through insect pollinators. After maturity, the seeds from the tagged male sterile plants are collected and will be used for producing male sterile lines again or for producing hybrid seeds.

The main difference between the hybrid seed production and the male sterile line maintenance is, during hybrid seed production the male fertile plants from the male sterile population are to be rogued off, while they are retained during male sterile line maintenance.

III. COTTON
Hybrid Seed Production (TCHB 213)

Parentage : TCH 1218 x TCB 209
(G.*hirsutum*) (G.*barbadense*)

For the seed production in an area of one acre, the female parent TCH 1218 is to be raised in 80 cents and the male parent TCB 209 in 20 cents.

Spacing

For female parent 4' x 2'
Male parent 3' x 2'

Synchronisation

Sowing of male parent should be advanced by 15 days. The male parent should be sown 5 meters away from the female.

Seed rate

Female parent : 800 g
Male parent : 200 g

Season

August. Dibble the seeds of male parent at 2 seeds/hill on 1st August and female parent on 15th August.

Emasculation and pollination

Emasculate and pollinate as far as possible in the buds appearing during the first six to eight weeks of reproductive phase to ensure good setting and development of bolls.

Restrict emasculation to each day evening from 3 to 6pm and pollination next morning between 9 AM to 1 PM.

Cover the male buds in the previous day evening with butter paper bag for their use in the next day.

Emasculated buds may be protected with butter paper bag. Tie a thread to the pedicel of the bud immediately after pollination.

Close the crossing programme after 9th week from commencement of crossing and flowers appearing subsequently are removed to facilitate proper development of crossed bolls.

Nip the top and side shoots to arrest further vertical and horizontal growth respectively.

Normally one flower from the male parent will cover 5 to 10 flowers of the female parent for crossing.

Exercise:

1. Compare the different hybrid seed production techniques followed in the crops like Rice, Sorghum, Maize, Cotton, Cumbu and Redgram.

12. Studies on segregating generations and maintenance of records

Maintenance of Records

1. Accession Register
2. germplasm Bank
3. Descriptive blank register
4. Cropping programme
5. Single plant selection register

Field Note Book

6. Row test
7. Replicated row test
8. Preliminary/Initial evaluation trial
9. Comparative yield/ yield evaluation trial
10. Multilocation I, II trials.
11. Quality observations Note book
12. Record of crosses
13. F1 generation
14. F2 segregation generation note book.

Accession Register

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

Proforma for Accession Register

Accession No. 1	Name of variety 2	Date of Receipt 3	Source of seed 4	Source No. 5	Pedigree record 6	Description of the material 7	How disposed to whom sent 8	Feed back information 9	Re-marks 10

Standard form of a Field Note Book

Each field note book should contain the following information.

A. Yield Trial

i) First Page

a) Number and title of the project
b) Season of raising the crop
c) Unit under which the trial is being conducted

ii) Second page

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

iii) Third Page

a) Plan of the experiment
b) Experiment details
1. Name of the experiment
2. Season
3. Number of variants
4. Design of the experiment
5. Replication
6. Size of the plot (Block/Plot/Row., etc.,)
7. Spacing (Between rows and within the row in cm)
8. Date of sowing/planting
9. Date of harvest
10. Name of the Principal Investigator

iv) Fourth page

Details of cultural practices followed for the plot/ field

a. Date of ploughing
b. Date of layout of the trial
c. Manurial schedule adopted
   Basal :
   Topdressing :
d. Irrigation schedules with date from life irrigation onwards
e. Plant protection schedules followed
f. Details of intercultural operations A (hoeing, weeding, and earthing up etc.,)
g. Date of harvest
h. Duration of processing till storage
i. Rainfall received during the crop growth
j. General remarks on the seasonal condition prevailed and its effects on crop growth including the occurrence of pests and disease.

v) Fifth page

One page for each variant per replications allotted.

The following information have to be recorded in each page.

1. Date of germination
2. Date of gap filling
3. Initial stand on
4. Date of first flowering
5. Date of general flowering
6. Date of harvest
7. Final stand
8. Wet weight of grain
9. Wet weight of haulms/ straw etc.,
10. Dry weight of produce after cleaning
11. Yield per ha in kg.

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

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

e.g.	Rice	: Date of earhead emergence in the main shoot number of tillers, : Date of earhead emergence in tillers and : Number of tillers.
	Cotton	: Number of sympodial branches : Number of monopodial branches
	Cumbu	: Date of emergence of female flowers : Date of emergence of male flowers : Number of tillers
	Sunflower	: Duration of flower opening etc.,

Generation study

This field note book will contain the following information.

a. Plan for the segregation generation
b. Details of the generation

1. Name of the generation study
2. Number of crosses
3. Details of the cross
   Cross No. Female parent, Male parent, Number of families, number of seed sown.
4. Length of row
5. Spacing (cm)
6. Date of sowing
7. Dates of harvest
8. Name of the Principal Investigator

Exercise

1. Observe the different records maintained at the Department of millets.
2. Observe the pedigree record maintained for different varieties of cotton.

13. Studies on different wild species in crop plants and wide hybridization

Wild species or wild genetic resources are the potential sources of desirable genes for various characters of crop plants. Wild species are nothing but the genetically related uncultivated species of cultivated ones. Wild species are believed to have evolved into the cultivated crops. They offer wide scope for studying the evolutionary process of creation of a cultivated species. Besides, the wild types usually abundant in nature for every crop promise to deliver to benefit the mankind through their genes transferred to the cultivated species by way of hybridization etc., make the latter to be more drought tolerant and pests and disease resistant. Here the important wild species of a few cultivated crops have been enlisted.

Crop	Cultivated species	**Ploidy level**	Wild species
1. Rice	*Oryza sativa* ***Oryza glaberrima***	2n = 2x = 24	*O.nivara* *O.rufipogon* *O.longistaminata* *O.officinalis*
		2n = 4x = 48	*O.minuta* *O.latifolia* *O.ridleyi* *O.longiglumis*
2. Wheat	*Triticum monococcum* *Triticum turgidum or T.dicoccum* *Triticum aestivum*	2n = 2x = 14	*T. dischasians* *T. tauschii*
		2n = 4x = 28	*T. timopheevi*
		2n = 6 = 42	
3. Barely	*Hordeum vulgare*	2n = 2x = 14	*H.comosum* *H.nuticum* *H.pusillum*
		4x	*H.capense* *H.secalinum*
		6x	*H.asixonicum* *h.lecblesi*
		2x & polyploids H. chilense	*H.bulbosum*
4. Maize	*Zea mays*	2n = 2x = 20	*Z.diploperennis*
		4x	*Z. perennis*
		2x and 4x Z. mexicana	*Tripsacum*

5. Sorghum	*Sorghum bicolor*	2n = 2x = 20	*S.propinqum*
		2n = 4x = 40 ***S.arundinaceum***	*S.halopense* *S.album*
6. Cotton	*Gossypium herbaceum* *G.arboreum*	2n = 2x = 26	*G.anomalum* *G.stuntrianum* *G.stocksii* *G.longicalyx* *G.bickii* *G.thurberi*
	G. birsutum *G.barbadense*	2n = 4x = 52	*G.armourianum* *G.tomentosum* *G.caicoense*
7. Groundnut	*Arachis bypogaea*	2n = 4x = 40	*A.syltvestris* *A.monticola* *A.villosulicarpa* *A.glaberata*
8. Sesamum	*Sesamum indicum*	2n = 26 = 52	*S.occidentale* *S.laciniatum* *S.prostratum*
9. Sunflower	*Helianthus annus*	2n = 34	*H.tuberosus* *H.petioloris* *H.gigantus*
10. Sugarcane	*Saccharum officinarum*	2n = 8x = 80	*S. robustum* *S.sinense* *S.spontaneum* *S.barberi*
11. Redgram	*Cajanus cajan*	2n = 2x = 22	*Rhynchosia*
12. Soybean	*Glycine max*	2n = 2x = 22	*G.soja* *G.tomentella*

Wide hybridization

Wild relatives of the cultivated crop species form an important reservoir of genetic variability for various economic characters such as disease and insect resistance, tolerance to abiotic stresses, increased biomass and grain yield and improved quality characters. This has lead to considerable interest in interspecific breeding for effecting transfer of desirable genes from wild species into related cultigens. When the donor and the recipient species are closely related, there is usually no genetic or ploidy barrier in the production of hybrid with a satisfactory level of chromosomal pairing. In such cases gene transfer can be and has been accomplished by conventional methods, such as hybridization and back crossing.

A few notable examples of the successful transfer of alien genes for improvement of cultivated cereals include that for rust resistance, grassy stunt resistance in rice, mildew and crown rust resistance in oats and increased biomass and grain yield in oats, pearl millet and sorghum. Unfortunately majority of the wild relatives of crop species have developed reproductive isolating mechanisms which may limit interspecific hybridization or inhibit genetic introgression through lack of adequate chromosomal meiotic pairing. Even where pairing occurs, linkage may restrict recombination between desirable and undesirable genes and prevent production of line with desired agronomic characters. Several barriers which operate at various levels prevent the successful gene transfer from wild into cultivated species. Recently significant advances were made in over coming these barriers, and various techniques of chromosomal manipulation and alien gene transfer were developed.

Exercise

1. Observe the different wild species for the following crops
   a. Rice, b. Cotton, c. Redgram, d. Forage crops
2. Comment on the morphological differences existing between the wild and cultivated species of the above crops.
3. Explain nobilization of Indian canes.

14. Estimation of heterosis

Heterosis is nothing but hybrid vigour which is defined as the increase in size (or) vigour of F_1 hybrid over its parents and is usually expressed in percentage.

Heterosis is expressed in three ways:

i. Relative heterosis = (F_1 performance over mid parental value)

$$di = \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100$$

ii. Heterobeltiosis = (F_1 performance over better parent value)

$$dii = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

iii. Standard heterosis = (F_1 performance over standard variety)

$$diii = \frac{\overline{F_1} - \overline{SP}}{\overline{SP}} \times 100$$

Exercise

In redgram 25 hybrids along with 10 parents were evaluated for heterosis on seed yield per plant. The genotypes were raised in RBD with three replications. Calculate the different types of heterosis and interpret the results.

Seed yield cg/ plant

S. No.	GENOTYPES	R I	R II	R III
1.	L_1	31.41	13.26	11.54
2.	L_2	13.78	8.18	8.05
3.	L_3	19.58	11.42	10.15
4.	L_4	29.35	14.77	9.79
5.	L_1	6.52	4.53	3.71
6.	T_1	28.54	12.84	13.54
7.	T_2	36.51	17.10	15.76
8.	T_3	25.41	18.05	19.80
9.	T_4	38.83	13.53	11.06
10.	T_5	34.15	17.55	21.93
11.	$L_1 \times T_1$	53.00	27.52	25.23
12.	$L_1 \times T_2$	48.60	27.47	24.45
13.	$L_1 \times T_3$	55.95	26.54	24.69
14.	$L_1 \times T_4$	35.47	15.82	10.10
15.	$L_1 \times T_5$	33.65	23.08	14.56
16.	$L_2 \times T_1$	31.43	15.55	12.93
17.	$L_2 \times T_2$	42.86	21.33	19.67
18.	$L_2 \times T_3$	26.67	12.57	16.12
19.	$L_2 \times T_4$	20.33	10.58	11.43
20.	$L_2 \times T_5$	34.19	14.26	9.11
21.	$L_3 \times T_1$	30.27	20.55	21.48
22.	$L_3 \times T_2$	49.52	19.07	16.85
23.	$L_3 \times T_3$	29.67	9.73	8.95
24.	$L_3 \times T_4$	30.17	21.86	15.93
25.	$L_3 \times T_5$	31.33	23.02	13.11
26.	$L_4 \times T_1$	25.83	10.89	8.54
27.	$L_4 \times T_2$	56.25	32.51	29.76
28.	$L_4 \times T_3$	17.67	14.06	7.83
29.	$L_4 \times T_4$	23.17	16.54	8.32
30.	$L_4 \times T_5$	25.52	9.92	7.51
31.	$L_5 \times T_1$	7.63	5.54	4.33
32.	$L_5 \times T_2$	36.32	17.83	11.23
33.	$L_5 \times T_3$	14.87	10.97	9.34
34.	$L_5 \times T_4$	15.03	9.33	6.28
35.	$L_5 \times T_5$	9.48	4.24	3.05

15. Screening methods – laboratory – field – for specific traits.

A. Screening techniques for pest and disease resistance

The different screening techniques for pest resistance are listed below.

1. Field Screening

The varieties to be evaluated or the segregating population to be screened for resistance may be raised in fields under natural infestation, either in endemic areas or by adopting techniques for increasing field infestation. Cultural practices such as closer spacing to create the most desirable humid microclimate within the crop, application of additional dose of nitrogen and irrigation to induce vegetative growth may be adopted. The test material may be sown or planted early, before the adult emerge in an area already infested in the previous season.

In case of insects such as plant hoppers, flies etc. that have a tendency to move at a rapid speed, fibre glass mesh cages may be kept on microplots and artificially reared insects released at a specified number per plant.

The insects population should be able to infest the plant population uniformly so that plants that escape infestation are not graded as resistant.

Highly susceptible plants can be interplanted as "spreader rows" along with rows of the test material. Insect attractants, such as fish meal for sorghum shoot fly may also be used to attract and increase the insect density in the field.

2. Green house screening

Screening the varieties or segregants in green houses providing conditions conductive for infestation is more rapid reliable than field screening. Special methods have been developed to increase the insect population to provide sufficient insect pressure for valid screening. Insects reared in the culture maintenance cages are released on plants raised in seed boxes kept in green house. Fibre glass screen cages are used for each seed box.

Depending on the crop on one hand and the insect pest on the other, the stage of the crop at which the insect is released, the stage of the insect, whether egg, larva or adult and the number of the insects population vary, as also the symptoms on the host to differential and grade the resistant plants from the susceptible.

3. Laboratory Screening:

Laboratory screening for resistance can also be done using plant tips or leaf discs and allowing forced or free choice feeding by insects as in the case of lucerne weevil.

4. Bioassay techniques

Bioassay techniques are also used to screen resistance to insect such as Heliothis spp and pink boll worm in cotton. Lyophilized square powder is incorporated in an artificial diet and dispensed into two-ounce plastic containers. Late first or early second instar larvae of Heliothis are weighed and placed in the diet cup. By periodic observations, larval survival, larval growth and percent pupation are recorded. Dates on fecundity and longevity of emerging adults are obtained to screen resistant types.

B. Screening Techniques for Diseases Resistance

The first step in a resistance breeding programme is to rapidly screen all the available genetic stocks, including the local land races, improved cuttings and exotic germplasms using empirical techniques in glass houses, or by field tests. An escape from disease is a hazard of breeding for resistant varieties. There are two major techniques to achieve the objectives.

1. Artificial inoculation

The artificial inoculation of test genotypes is necessary to obtain a more uniform disease – epidemic than world occur naturally such. A uniformity facilitates comparison among disease genotypes. For a successful screening, an adequate amount of inoculum is necessary, since selecting for resistance in the presence of an unadequate amount of disease create an instant artifact. Among few methods developed, three main procedures of artificial inoculation are generally employed.

i) Spraying the spore suspension on test genotypes – using an autominyor the aqueous suspension of the pathogens propagules is sprayed. High pressure spraying is most productive.

ii) Injection of spore suspension into the plants surface or into the intercellular air spaces of a leaf with the help of a hypodermic needle. A single or multineedle pricking may be employed.

iii) Immersion of seedlings of test genotypes in a spore suspension before transplanting them into fields. Since the host tissue becomed water soaked, they predispose themselves to disease.

Though spore – suspension in sterile water (Inoculum) is often used, successful inoculum of many pathogens can be achieved only if dry spores are employed. Spores are damaged quickly if they became too wet. For good results, any one of these three methods of artificial inoculation can be used. Depending upon the convenience and crop materials the procedure may vary from one disease to another and from one crop to another. But similarities are not ruled out.

2. Artificial epiphytotics

The manual operation of artificial inoculation can be dispensed with once a sick plot or disease nursery is created. Artificial creation of such an epiphytotic is necessary

because the occurrence of natural disease is subject to chance. This can be attained by seeding spores in the soil (in case of only soil borne disease caused by facultative parasites) either through growing a highly susceptible genotype in a plot for successive years, or through mixing infected debris in the soil of that plot, prior to sowing the test genotypes. Maintenance of requisite humidity is essential for high effectiveness.

i) The epiphytotic should be intense developed at both national and international levels. The international wheat rust nursery at Mexico established since 1957 is an outstanding example of such an international collaboration.

ii) **Frequency of exposure:**
The test genotypes can be subjected to artificial inoculation or sick plots once or more than area for desired results. Two tests are suggested.

a. Polycyclictest:

Exposure that initiates the natural occurrence of diseases with a recurrent infection cycle attacking a crop on the farmers field. The artificially created sick-nursery is the best form of such polycyclic tests, through repeated artificial inoculations can also achieve the same results.

b. Monocyclictests:

Exposure only once by artificial inoculation, preferably under closed/ controlled condition, such as green houses.

However in actual practice neither of the two tests are employed by breeders. A continous monocyclic test is applied since the moderately resistance cultures are subject to the monocyclic test everyday and which is conductive to injection.

Testing the host plants either by artificial inoculation or under sick nurseries is the beginning of the screening process. Next comes how to measure the intensity of diseases.

3. Measurement of the intensity of disease infection.

There are two methods to assess the disease intensity depending upon the inheritance of the disease.

i) Qualitative Method:
If reflects the response type representing a class but not the magnitudes in monogenic disease control.

ii) Quantitative Method:
This quantifies or determines the degree of infection in terms of grades or percentages or indices.

The following are commonly used.

i) Simple usual scoring

Each plant or group of plants may be assigned a numerical score (grade) for the severity of symptoms as under (Russell, Q78)

O = nosymptoms, 1 = very mild symptoms, 2 = mild symptoms, 3 = severe symptoms and 4 = very severe symptoms.

Pate and Harvey (1954) also used scores from 0-5 with O for immunity and 5 for very heavy infection of *Helminthosporium maydis*.

ii) Grading system of Harsfall and Barrat (1954)

At below 50 percent infection, the eye sees the amount of disease tissues and at above 50 percent infection, it sees the amount of disease free tissues. The simple usual scoring does not take care of this principles. However, Hargfall and Bassats grading systemic based on 50 percent as mid part as under.

Grade	1	=	0 % Kill	Grade	7	=	50-75 %
"	2	=	0-31 %	"	8	=	75-87 %
"	3	=	3-6 %	"	9	=	87-94 %
"	4	=	6-12 %	"	10	=	94-97 %
"	5	=	12-25 %	"	11	=	97-100 %
"	6	=	25-50 %	"	12	=	100 %

Meangrade = grade reading ÷ number of reading.

Grade indicates 0 percent disease, while grade 12 means 100 percent disease infection.

iii) Ullstrip (1944) Diagrammatic Standard:

0.5 = Very light infection, one or two restricted lesions on lower leaves.

1.0 = Slight infection a few scattered lesions on lower leaves.

2.0 = Light infection moderate number of lesions on lower leaves.

3.0 = Moderate infection, abundant lesions on lower leaves, a few on middle leaves.

4.0 = Heavy infection, lesions abundant on lower and middle leaves extending to upper leaves.

5.0 = Very heavy infection, lesions abundant on all leaves plants may be prematurely killed.

A rating of (2.0) is generally a satisfactory lead for breeding purposes.

iv) Infection Index of villareal and Lantican (1965)

0 = No infection (N)

1 = Very slight infection (Vs) very few lesions on lower leaves.

2 = Slight infection (5) moderate number of lesions on loweor leaves.

3 = Moderate infection (M); abundant lesions on lower leaves and a few on middle leaves.

4 = Heavy infection (H); lesions abundant on lower and middle leaves, extending to upper leaves.

5 = Very heavy infection (VH); lesions abundant on all leaves, plants may be prematurely killed.

v) A Modified Infection Index

The All India Millet workers conference held at Gwalior March 1976 recommended a modified version of the above grading system for scoring downy mildew incidence in pearl millet. According to this scheme, each plant is assigned a security scale/ score as under:

1 = No symptom
2 = Symptom on nodal tillers only (green ear to blind tillers (5 % infection)
3 = Symptoms on main tillers (green ear or blind tillers) three or more productive heads (10% infection)
4 = Symptoms on most main tillers so that there are only one or two productive heads (30% infection)
5 = Symptoms on all tillers ; No productive heads (35% infection)

Drought tolerance

If high yielding varieties involved for irrigated situations are screened rigorously under upland conditions, it would ensure adaptability of selected materials to local conditions as well as a high yield potential.

Many practical agonomists and breeders have realized that the best yielding varieties of irrigated rice are not necessarily the best in rainfed, drought prone conditions.

The following characters should be taken into account while screening for drought resistance.

1) **Screening for growth – early maturity is preferred.**

2) **Screening for seedling vigour**

3) **Screening for height**

4) **Screening for root characteristic:**
Root characteristic such as size, morphology, depth, length, density and function are important in maintaining relatively high leaf water potential against evapotranspirational demand. A deep root system is an important component of drought resistant because it enables the plant to export water in the deep soil layers. Of all the attributes, root length density is probably the major operative factor that largely determines the extent to which roots can extract water from the adjacent soil.

5) **Screening for leaf rolling**

6) **Screening for leaf water**
Variations in leaf water potential among cultivars or strains under moisture stress up to 10-13 bars between extreme rice genotypes have been found.

7) **Screening for osmotic adjustment**

Osmotic adjustment generally considered as the net increase in ultracellular solutes, usually occurs in response to various environmental stresses.

8) **Screening for translocation of photosynthesis.**
Screening for relative ability many genotypes for translocation efficiency as a measure of waterstress tolerance has been suggested.

9) **Screening for amino-acid accumulation**
Certain amino-acids increase dramatically under stress and screening for them has been suggested as a means of evaluating drought resistance. Observations suggest that proline could provide metabolic advantages within limits of water stress.

10) **Screening for stomatal behaviours**
Actions of stomata provide important mechanisms to reduce water loss during stress. Higher leaf water potential can be maintained by stomatal closure.

11) **Screening for cuticle characteristic**
Cuticular permeability or transpiration is affected by thickness of cuticular layer and subsequently is influenced by the amount composition and physical configuration of epicuticular wax deposits.

12) **Screening for xylem, vessel structure**
Dryland varieties generally have larger xylem vessels in seminal roots than semidwarf. Larger vessels decrease root axial resistance to upward water transport.

13) **Screening for desiccation tolerance ability.**
The development of simple screening techniques based an exposure of leaf portion or seedlings to heat shows wide variation in dehydration tolerance among species and cultivars.

Exercise

1. Visit to cytogenetics glass house, observe the screening procedure for pest resistance and comment critically.

16. Procedure for marker assisted selection

Marker Assisted selection is a technique which is followed when an important trait that is difficult to asses is tightly linked to a trait that is easily measured. For example, in rice a gene for resistance to brown plant hopper (bph) is closely linked to a gene specifying purple coleoptile color in some traditional rice varieties grown in north east India. When a resistant plant with a purple coleoplite is crossed with a susceptible plant with a green coleoptile, more than 95% of F_2 plants showing purple coleoptile are also resistant to BPH. In this case, coleoptile color is a morphological marker which is used to assist the selection for BPH resistance.

Unfortunately few morphological markers are known. They tend to be specific for particular rice varieties and most morphological markers are mutations which are deleterious to rice plants. Homozygous locus is indistinguishable from a heterozygous one, when a dominant allele is involved. Moreover, the usefulness of the approaches limited to traits controlled by single major gene, it doesnot apply to many agronomically important traits that are governed by many unlinked genes.

The advent of molecular markers has enormously increased the power of MAS. The most commonly used DNA markers are RFLPs. Other kinds of DNA markers have been developed recently. In MAS, target genes are detected based on the genotype as determined by DNA markers, and not on the phenotypic expression of the genes.

The selection based on the DNA marker permits selection of target gene unless the selected individual carries a recombinant chromosome. If the recombination frequency between DNA marker and target genes doesnot change from gene mapping population to breeding population, it is possible to select the target gene based on DNA marker with a predictable rate of accuracy.

The procedure of PCR based MAS is to use the PCR techniques to generate markers which are linked to target genes.

To set up a working laboratory for PCR-based MAS, the following equipments are needed.

Equipment for PCR based MAS laboratory

Sl. No.	Item	Quantity	Estimated costs (in US $)
1.	Microcentrifuge	1	1400-1800
2.	DNA thermal cycler machine	1	4000-9000
3.	Electrophoresis system	1	1000-1500
4.	Fotodyne camera	1	2000
5.	Micro wave oven	1	500
6.	Hot plate stirrer	1	300

7.	Auto clave	1	5000
8.	Freezer (-20^{O}C)	1	3000
9.	Refrigerator	1	1000
10.	Balance	1	2000
11.	pH meter	1	500
12.	Laminar flow hood	1	5000
Total			**25,700-31,600**

After a MAS laboratory is constructed, the procedure involves the following.

1. Rapid isolation of DNA suitable for PCR analysis
2. DNA amplication through PCR
3. Restriction manipulation of PCR products.
4. Gel analysis of PCR products
5. Identification of plants carrying target gene based on PCR markers.

Exercise

1. Write the principles of PCR
2. List out different types of molecular markers.
3. Write the significance of PCR.