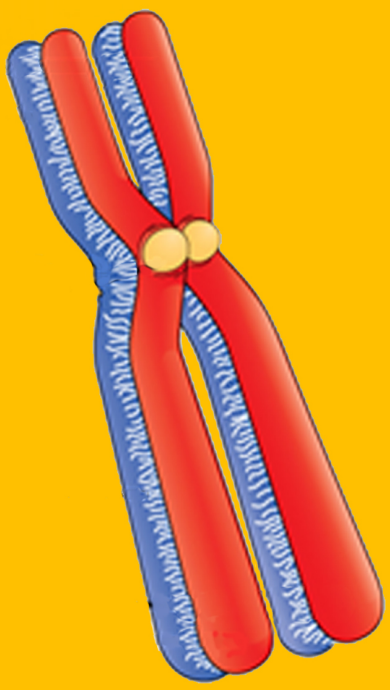
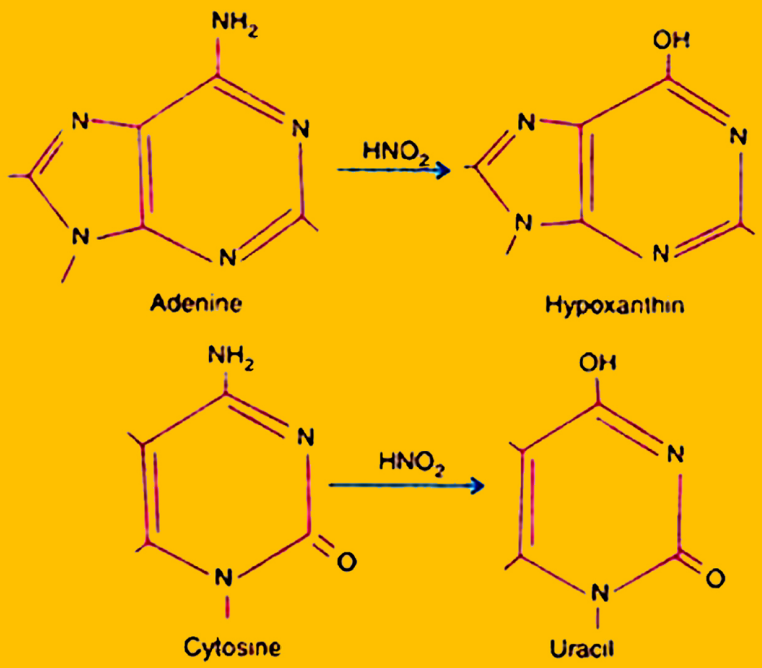
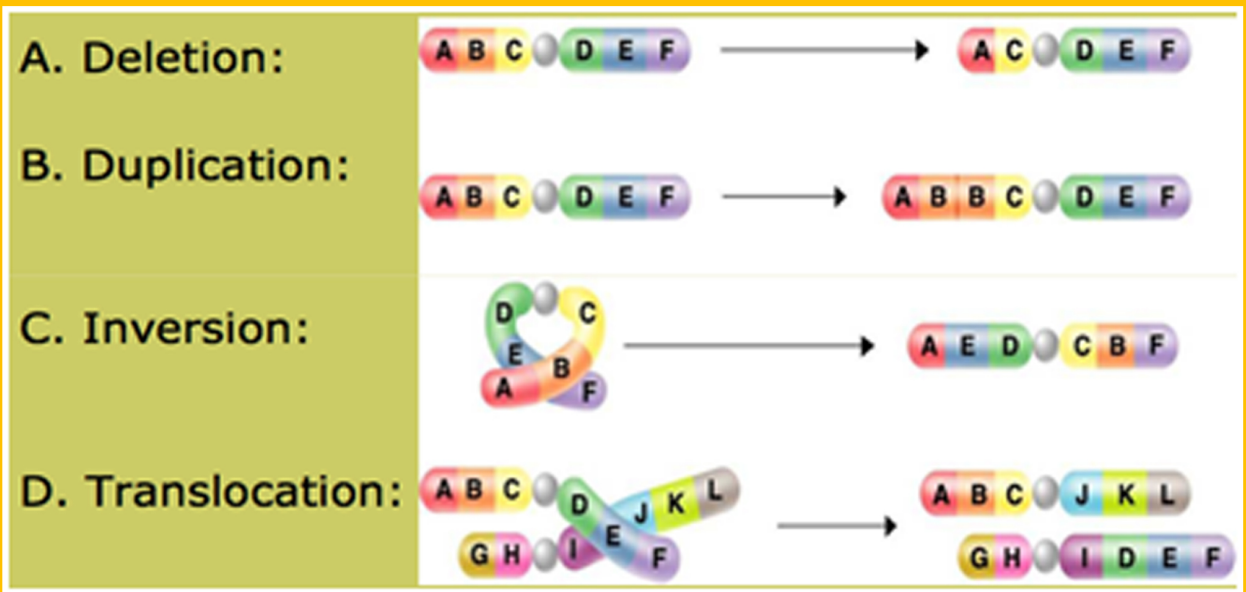
MSCBOT-507

M. Sc. II Semester

CYTOGENETICS AND PLANT BREEDING







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SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

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BLOCK-1- INTRODUCTION TO PLANT BREEDING

UNIT-1 GENETIC VARIABILITY

1.1 OBJECTIVES

1. To study genetic variance.
2. To study gene action and various factors affecting it.
3. To study genetic variance in plant breeding.

1.2 INTRODUCTION

In polygenic inheritance, segregation occurs at a large number of loci affecting a trait. The polygenic variation present in a plant population is of three types, viz., phenotypic, genotypic and environmental. Phenotypic variability is observable. It includes both genotypic and environmental variation and therefore, is also called variation. It changes under different environmental conditions and is measured in terms of phenotypic variance.

Genotypic variation refers to genetic or inherent variability which remains unaltered by environmental conditions. This type of variability is more useful to a plant breeder for exploitation in selection or hybridization. It is measured in terms of genotypic variance and consists of additive, dominance and epistatic components. The genetic properties of a population are determined by the relative magnitudes of the components of variance. By knowing the components of variance, one may estimate the relative importance of the various determinants of phenotypes. Phenotypic value of quantitative traits is expressed as:

P (Phenotypes) = G (Genotypes) = E (Environment)

The phenotypic value is variable because it depends on genetic differences among individuals as well as environmental factors and the interaction between genotypes and environment called (G=E interaction). Total variance of quantitative trait may be mathematically expressed as follows:

$$VP = VG + VE + VGE$$

Where VP= total phenotypic variance of the segregating population, VG= genetic variance, VE= environmental variance and VGE= variance associated with the genetic and environmental interaction. The genetic component of variance may be further partitioned in to three components as follows:

$$VG = VA + VD + VI$$

Where VA= additive variance (variance from additive gene effects), VD= dominance variance (variance from dominance gene action), and VI= interaction (variance from interaction between genes). Additive genetic variance (or simply additive variance) is the variance of breeding values and is the primary cause of resemblance between relatives. Hence VA is the primary determinant of the observable genetic properties of the population and of the response of the population to selection.

Environmental variation is entirely due to environmental effects and varies under different environmental conditions. This uncontrolled variation is measured in terms of error mean variance. The variation in pure lines and their F_1 is non-heritable (environmental). To obtain environmental variance, individuals from same genotype are used. An inbreed line (essentially homozygous) consist of individuals with same genotype. An F1 generation from a cross of two inbred lines will be heterozygous but genetically uniform. The variance from the parents and the F1 may be used as a measure of environmental variance (VE). In sum, variances from additive, dominant, and environmental effects may be obtained as follows:

VP1=E; VP2=E; VF1=E
VF2=1/2 A+ 1/4D+ E
VB1= 1/4 A+1/4D+ E
VB2= 1/4A+1/4D+ E
VB1+ VB2=1/2 A+1/2 D+2E

Genetic variance refers to the heritable portion of phenotypic variance. In crop improvement programme only the genetic component of variation are important because only this component transmitted to next generations. Genetic variance has been classified by Fisher (1918), Wright (1935) and Mather (1949). Fisher has divided the genetic variance into three components, *viz.*, additive, dominance and epistatic variances. Wright classified genetic variance into two fractions, *viz.*, additive variance and non-additive variance. Mather also divided the genetic variance into two parts, *viz.*, heritable fixable and heritable non-fixable (Table-1).

TABLE 1: Classification of genetic variance according to various authors

S. No	Classified by	Types of genetic variance	Brief description / definition
1.	Fisher 1918	Additive variance Dominance Variance Epistatic variance	Average effect of genes on all segregating loci. Deviation from mean value due to intrallelic interaction. Deviation from mean value due to non-allelic interaction. It is of 3 types, *viz.*, AA, AD and DD.
2.	Wright 1935	Additive variance Non-additive	Same as given by Fisher. It includes dominance and epistatic variances.
3.	Mather 1949	Heritable-Fixable Heritable-Non fixable	Includes additive variance and AA component of epistasis Includes dominance variance and AD and DD types of epistasis.

A brief description of additive, dominance and epistatic variances is presented as follows:

A. Additive variance

Additive variation is the total effect on a trait stemming from one or more gene loci. Each locus contributes to the trait in a measurable way. It refers to that portion of genetic variance which results due to average effects of genes on all segregating loci. Thus it is the component which

arises from differences between two homozygotes of a gene, *i.e.*, *AA* and *aa*. This results in deviance from the mean phenotype due to inheritance of a particular allele and its relative effect on phenotype. It measures the magnitude to which individual phenotype differences can be prophesied due to additive effects of allelic substitutions.

Main features of additive genetic variance are given below:

1. It is a measure of additive gene action. The additive genes show lack of dominance, i.e., intermediate expression.
2. Additive genetic variance occurs due to genes which show an additive effect on the quantitative trait.
3. Additive genetic variance is associated with homozygosity and, therefore it is expected to be maximum in self-pollinating crops and minimum in cross-pollinating crops.
4. Additive variance is fixable and, therefore, selection for traits governed by such variance is very effective. Existence of additive variance is a pre-requisite for improvement through selection because this is the only variance which responds to selection.
5. Additive variance is required for estimation of heritability in narrow sense and response to selection is directly proportional to narrow sense heritability.
6. Breeding value of an individual is measured directly by the additive gene effects. The general combining ability (*gca*) effect of a parent is a measure of additive gene effects.
7. Additive genetic variance gets depleted proportionate to the improvement made by selection.
8. In natural plant breeding populations, additive variance is the predominant one closely followed by dominance variance.
9. Additive gene action is the chief cause of resemblances between relatives and progress by selection is directly proportional to the degree of resemblance between the parent and its progeny. Thus additive gene action is a measure of breeding value of a genotype.
10. Transgressive segregation is the result of additive gene action. Transgressive segregants, which fall outside the range of parents, are obtained due to fixation of dominant and recessive genes in separate individuals. Such segregation occurs when the parents are intermediate to the extreme values of the segregating population.

B. Dominance variance

Dominance genetic variance is associated with dominant gene actions which cover the influence of the recessive alleles at the particular locus. It refers to the deviation from the additive scheme of gene action resulting from intra-allelic interaction, i.e., interaction between alleles of the same gene or same locus. It is due to the deviation of heterozygote (*Aa*) from the average of two homozygotes (*AA* and *aa*). Main features of dominance variance are briefly discussed below:

1. It is a measure of dominance gene action. Such genes show incomplete dominance, complete dominance or over dominance. In other words, the heterozygote (*Aa*) does not represent mean value of two parents for a particular character. The parent to which the heterozygote approaches more closely for a particular trait is known as dominant parent and the other as recessive parent.

2. Dominance variance is associated with heterozygosity and, therefore, it is expected to be maximum in cross-pollinating crops and minimum in self-pollinating species.
3. Dominance variance is not fixable and, therefore, selection for traits controlled by such variance is not effective.
4. Dominance variance is the chief cause of heterotic hybrid vigour.
5. Specific combining ability variance is the measure of dominance variance in diallel, partial diallel and line × tester cross analysis.
6. Dominance variance gets depleted through selfing or inbreeding.
7. In natural breeding populations, dominance variance is always lesser than additive variance.

C. Epistatic variance

Epistatic genetic variance occurs due to statistical interaction among loci, i.e., gene-by-gene interaction. It refers to the deviation from additive scheme as a consequence of inter-allelic interaction, i.e., interaction between alleles of two or more different genes or loci. Main features of epistatic variance are given below:

1. Epistatic variance is a measure of epistatic gene action.
2. Epistatic variance is of three types, viz. additive × additive, additive × dominance and dominance × dominance as defined below:

(a) ***Additive × additive.*** It refers to interaction between two or more loci each exhibiting lack of dominance individually. It is denoted as $A \times A$ and is fixable.

(b) ***Additive × dominance.*** It refers to interaction between two or more loci, one exhibiting lack of dominance and the other dominance individually. It is denoted as $A \times D$ and is non fixable.

(c) ***Dominance × dominance.*** It refers to interaction of two or more loci, each exhibiting dominance individually. It is represented as $D \times D$ and is non-fixable.

3- First type of epistasis is fixable and, therefore, selection is effective for traits governed by such variance. The last two types of epistatic variances are unfixable and, therefore, heterosis breeding may be rewarding for traits exhibiting such variance.

4- In case of generation mean analysis, the epistatic gene interactions are classified on the basis of sign of (h) and (l) into two types only, viz. complementary and duplicate. When (h) and (l) have the same sign, it is called complementary type and when (h) and (l) have opposite sign, it is termed as duplicate type of epistasis.

5- In natural plant breeding populations, epistatic variance has the lowest magnitude.

1.3 STUDY OF GENETIC ACTION AND FACTOR AFFECTING ITS GENE ACTION

Gene action refers to the behaviour or mode of expression of genes in a genetic population. Knowledge of gene action helps in the selection of parents for use in the hybridization program and also in the choice of appropriate breeding procedure for the genetic improvement of various quantitative characters. Hence insight into the nature of gene action involved in the expression of

various quantitative characters is essential to a plant breeder for starting a judicious breeding program. Main features of gene action are briefly presented below:

1- Gene action is measured in terms of components of genetic variance or combining ability variance and effects.
2- Depending upon the genetic variance, gene action is of three types, *viz.* additive gene action, dominance gene action and epistatic gene action. Dominance and epistatic gene actions jointly are referred to as non-additive gene action.
3- Gene action can be studied with the help of various biometrical techniques such as diallel analysis, partial diallel cross, triallel analysis, quadriallel analysis, line × tester analysis, generation mean analysis, biparental cross and triple test cross analysis.
4- Gene action is affected by various factors.

1.3.1 Factors affecting gene action

Since genetic variances are used as measures of gene action, all those factors which affect estimates of genetic variance also affect gene action. Such factors include, type of genetic material, mode of pollination, mode of inheritance, existence of linkage, sample size, sampling method and method of calculation. These are briefly described below:

A. Type of Genetic material

The magnitude of gene action is largely governed by the type of genetic material used for the study. In an F_2 or advanced generation of a cross between two pure lines, the genetic variance includes additive, dominance and epistatic components. But in case of homozygous lines, the genetic variance is entirely of additive and additive-epistatic types. Thus in terms of genetic variance, self and cross pollinated species differ primarily in the relative magnitude of dominance component. In other word in homozygous genotypes, the genetic variance is entirely of additive and additive-epistatic types, while in the segregating populations both additive and non-additive types of gene actions are present (Table 2).

In F_2, the phenotypic variance has 1/2 D (additive) and 1/4 H (dominance) components. In a random mating population with no epistasis and zero inbreeding, the covariance between a parent and its offspring is 1/2 *VA*; the covariance among half-sibs is 1/4 *VA;* and the covariance among full-sibs is 1/2 *VA* + 1/4*VD*. These relationship changes with different levels of inbreeding in the population.

The nature of gene action for important agronomic characters in almost all the crops is mainly of additive type. The non-additive type of gene action also exists in nearly all crops and for many important traits, but is generally smaller in magnitude than additive components.

TABLE 2: Gene action in different types of genetic materials

Genetic material	Type of gene action

(a) Self-pollinated species	
1- Pure line variety	Additive but no genetic variation
2- Mass selected variety	Additive and additive epistasis
3- Multilines	Additive and additive epistasis
4- Varietal blends	
(b) Cross-pollinated species	
5- Composite variety	Additive, dominance and epistasis
6- synthetic variety	Additive, dominance and epistasis
7- Random mating population	Additive, dominance and epistasis
(c) Both self and cross-pollinated species	Non-additive and no genetic variation
8- F_1, Hybrid	Additive, dominance and epistasis
9- F_2 population	

B. Mode of Pollination

The gene action is greatly influenced by the mode of pollination of a plant species. The additive gene action is associated with homozygosity and, therefore, it is expected to be optimum in self pollinated species. The non-additive gene action is associated with heterozygosity and therefore, it is expected to be more in cross-pollinated species and minimum in self-pollinated crops. Inbreeding enhances the amount of additive genetic variance in a population due to increase in homozygosity by way of gene fixation. On the other hand, out breeding increases the proportion of non-additive genetic variance by way of creating heterozygosity in a population. Thus gene action changes with the mode of pollination (Fig.1). In cross-pollinated species, selfing or inbreeding leads to conversion of non-additive gene action into additive by way of converting heterozygotes into homozygotes. With single gene, more than ten generations of selfing are required for complete conversion of heterozygotes into homozygotes.

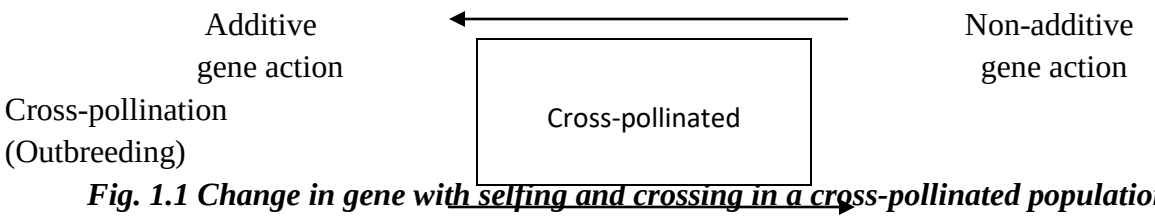

Fig. 1.1 Change in gene with selfing and crossing in a cross-pollinated population

C. Mode of Inheritance

Some characters are governed by one or few genes. Such characters are known as qualitative character or oligogenic characters. On the other hand, some characters are controlled by several genes. Such characters are referred to as quantitative or polygenic characters. Thus inheritance is of two types, *viz*. oligogenic and polygenic. Polygenic characters are governed by both additive and non-additive types of gene actions, though the additive gene action is predominant in the

expression of such characters. On the other hand, oligogenic traits are primarily governed by non-additive types of gene action (dominance and epistasis). In case of oligogenic traits, epistatic variance is of widespread occurrence, but comparable evidence for polygenic traits is meagre.

D. Existence of Linkage

The existence of linkage also affects the gene action. Linkage influences gene action by causing an upward or downward bias in the estimates of additive and dominance genetic variances. There are two phases of linkage, viz. coupling and repulsion. In case of coupling phase, there is linkage either between dominant genes (*AB*) or between recessive genes (*ab*). The repulsion phase refers to linkage between dominant and recessive genes (*Ab /aB*). High frequency of coupling phase (*AB/ab*) causes an upward bias in the estimates of additive and dominance variance. An excess of repulsion phase linkage (*Ab/aB*) leads to upward bias in dominance variance and downward bias in the additive variance (Table 3). Linkage disequilibrium can be reduced by random mating of population. In other words, linkage can be broken by repeated intermating of randomly selected plants to segregating populations. The number of intermating generations required for breaking the linkage depends on the closeness of the linkage.

TABLE 3: Effect of linkage of genetic variances

Type of linkage	**Upward bias in**	**Downward bias in**
Coupling phase	Additive variance	-
(*AB/ab*)	Dominance variance	-
Repulsion phase (*Ab/aB*)	Dominance variance	Additive variance

E. Sample Size

Estimates of genetic variance are influenced by the sample size on which the computation is based. Sample size should be adequate to obtain consistent and meaningful results. Small sample may not provide estimates of sufficient reliability. However, sample size may vary with the magnitude of genetic variability present in the genetic population under study. If the estimates are based on the entire population, it will give the true genetic variance of that population, but evaluation of entire population is not practically possible. Large sample size will give estimates of genetic variance nearer to the population mean, while small sample may give biased estimates.

F. Sampling Method

There are two main sampling methods, viz. random and biased sampling. The random sampling method generally provides true estimates of genetic variance and hence of gene action. The biased sampling on the other hand, will not give representative estimates of genetic variances and thereby gene action. Hence, the material to be evaluated should be a random sample of all possible genotypes present in the population.

G. Method of calculation

Several biometrical techniques are used for the estimation of genetic variance. The estimates of genetic variance obtained by various methods will vary to some extent. Moreover, use of some mating designs is based on certain genetical assumptions to obtain valid estimates of genetic variance. Failure to meet one or more of these assumptions may result in biased estimates of genetic components of variance.

H. Method of computation

Heritability is estimated by several methods that use different genetic populations and produce estimates that may vary. Common methods include the variance component method and parent offspring regression.

1.4 STUDY OF MALE STERILITY IN PLANTS

1.4.1 Role of genetic variance in plant breeding

Knowledge of gene action is useful to a plant breeder in three principal ways, *viz.* (1) in the selection of parents for hybridization, (2) in the choice of breeding procedures for the genetic improvement of various quantitative characters and, (3) in the estimation of some other genetic parameters. These are briefly described below:

A. Selection of Parents

Selection of parents for hybridization is an important step in plant breeding. Good generally combining parents can be identified by combining ability analysis. In self pollinated species, good general combining parents can be used in the hybridization programme for obtaining superior segregants in the segregating generations and in cross pollinated species such parents can be used for the development of synthetic and composite varieties.

B. Choice of Breeding Procedure

The inheritance of yield and most of the yield contributing characters is polygenic in nature and displays continuous variation. The choice of appropriate breeding procedure depends on the type of gene action involved in the expression of these characters in a genetic population (Table 5). Additive genetic variance is a pre-requisite for genetic gain under selection, because this is the only genetic variance which responds to selection. Additive genetic variance gets depleted proportionate to the improvement made by selection. In other words, genetic improvement through selection is achieved at the expenses of additive genetic variance. In pure line selection, additive genetic variance is completely depleted. That is why further improvement through selection is not possible in a pure line population. In pure lines, additive genetic variance is regenerated over a period of time by recombination and mutation.

If there is preponderance of additive gene action, reliance should be placed on mass selection and progeny selection in self pollinated species and synthetic and composite breeding in cross pollinated species.

TABLE 4: Breeding procedures in relation to gene action

Type of gene action	Breeding procedure to be adopted	Possible outcome
(a) Self-pollinated species		
1. Additive	Pure line selection	Pure line variety
	Mass selection	Mass selected variety
	Progeny selection	New variety
	Hybridization and selection	Superior segregant or new variety
2. Non-additive	Heterosis breeding	Hybrid variety
(b) Cross-pollinated species		
1. Additive	Recurrent selection for *gca* Synthetic breeding Composite breeding	Population improvement synthetic variety Composite variety
2. Non-additive	Heterosis breeding Recurrent selection for *sca*	Hybrid variety Population improvement
3. Both additive and Non-additive	Reciprocal recurrent selection	Population improvement

Non-additive gene action is a prerequisite for launching a heterosis breeding programme. If there is preponderance of non-additive gene action, the breeding objective should be towards development of hybrids for commercial purpose. If both additive and non-additive gene actions are of equal magnitude, population improvement programme cross-pollinated species, various types of recurrent selections are used depending upon the relative importance of gene action. Recurrent selection for general combining ability is effective with additive gene effects; recurrent selection for *sca* makes use of non-additive gene effects; and reciprocal recurrent selection utilizes both additive and non-additive gene effects.

C. Estimation of other Genetic Parameters

Genetic variances which are the relative measures of gene action are used for working out various genetic parameters. For example, additive genetic variance is required for the estimation of heritability in narrow sense and response to selection is directly proportional estimation of heritability in narrow sense and response to selection is directly proportional to narrow sense heritability.

In intermating population, additive genetic variance is never exhausted due to self conversion of non-additive genetic variance into additive one. This type of conversion takes place due to fixation of heterozygotes into homozygotes. In self-pollinated species additive genetic variance is in abundance in segregating generations and mixtures of several different pure lines. It is also present in adapted populations of out breeders. Thus additive genetic variance is of universal

occurrence in plant breeding populations. Non-additive variance also exists, but is generally smaller in magnitude than additive one. In natural plant populations, additive genetic variance is predominant, which is closely followed by dominance variance. Epistatic variance is the lowest in magnitude

1.5 SUMMARY

All genes are expressed in an environment (Phenotypes=Genotypes X Environment effects). However, quantitative traits tend to be influenced to a greater degree than qualitative traits. Genotypic variance divided in to three components: (1) a part due to the average effects of genes (now called additive genetic variance), (2) a part due to allelic interactions of genes that is called dominance variance and, (3) a part due to nonallelic interactions of genes that is called epistatic variance. A sound plant breeding program will include the use of information on the relative importance of these components. Selection of the best breeding procedure based on knowledge of the components of genetic variance must logically be expected to give the maximum genetic progress towards the objectives of a breeding program.

1.6 GLOSSARY

Additive × Additive Epistasis: Interaction between two or more loci each exhibiting lack of dominance individually. It is denoted as *AA* and is fixable.

Additive × Dominance Epistasis: Interaction between two or more loci, one exhibiting lack of dominance and the other dominance. It is denoted as *AD* and is non-fixable.

Additive variance: Average effect of genes on all segregating loci. Such genes show lack of dominance, *i.e.*, intermediate expression.

Character: Any property of an individual showing heritable variation. It includes morphological, physiological, biochemical and behavioral properties.

Dominance × Dominance Epistasis: Interaction of two or more loci, each exhibiting dominance individually. It is represented as *DD* and is non-fixable.

Dominance Variance: Deviation from the mean value due to intra-allelic interaction.

Environmental Variation: The non-heritable variation which is entirely due to environmental effects and varies under different environmental conditions.

Epistatic Variance: Deviation from the mean value as a consequence of non-allelic interaction *i.e.*, interaction between two or more genes. It is of three types *viz*., additive × additive, additive × dominance and dominance × dominance.

F1: Its full name is first offspring (filial generation). It is the first generation obtained by hybridization. The next and subsequent generations are referred to as F2, F3, *etc.*

F2: It is the second generation obtained on self fertilization of Fl.

F3: It is the third generation obtained on selfing of F2 hybrids.

Fixable gene Action: Type of gene action that can be fixed as a true breeding line. It includes additive gene action and additive × additive type of Epistasis.

Gene Action: Mode of expression of gene in a genetic population.

Genetic Variation: Heritable portion of total or phenotypic variance. It is of three types, *viz*., additive, dominance and Epistatic variances.

Genotype: The genetic makeup of an organism determined by the particular combination of alleles at one or more specific locations (loci) on one or more paired chromosomes.

Genotypic Variation: The inherent or genetic variation which remains unaltered by environmental changes.

Heterozygous: Carrying two different alleles for a particular gene.

Homozygous: Homozygous having the same allele at the same locus on both members of a pair of homologous chromosomes. Homozygous also refers to a genotype consisting of two identical alleles of a gene for a particular trait. An individual may be homozygous dominant (AA) or homozygous recessive (aa). Individuals who are homozygous for a trait are referred to as homozygotes.

Non-additive Gene Action: Joint effects of dominance and epistatic gene action.

Nonfixable Gene Action: Type of gene action that cannot be fixed as a true breeding line. It includes dominance gene action and additive × dominance and dominance × dominance types of epistatic gene actions.

Oligogenic Trait: Characters which are controlled by one or few genes each having detectable individual effect and exhibit discontinuous variation.

Phenotype: A phenotype is an individual's observable traits, such as height, eye color, and blood type. The genetic contribution to the phenotype is called the genotype. Some traits are largely determined by the genotype, while other traits are largely determined by environmental factors.

Phenotypic Variation: The total variability which is observable.

Plasmid: A plasmid is a small, often circular DNA molecule found in bacteria and other cells.

Polygenic Traits: Characters which are governed by several genes each having small individual effect and exhibit discontinuous variation.

Quantitative character: Character under complex genetic control. *i.e*. governed by many genes (polygenes) are known as quantitative characters. e.g. grain yield, drought tolerance, *etc*.

Qualitative characters: Character under single genetic control. *i.e*. Governed by one or few genes (oligogenes) are known as qualitative characters, e.g. flower colour, fruit shape.

Recessive: An allele that produces its characteristic phenotype only when the paired allele is the same; will be masked if a dominant allele is present. Trait expressed in people who are homozygous or hemizygous for a particular gene, but not in those who are heterozygous for the gene.

Trait: A trait is a specific characteristic of an organism. Traits can be determined by genes or the environment, or more commonly by interactions between them. The genetic contribution to a trait is called the genotype. The outward expression of the genotype is called the phenotype.

1.7 SELF- ASSESSMENT QUESTIONS

1.7.1 Very Short Answer Type Questions

1. What is gene action?
2. What is additive gene action?
3. What is Epistatic variance?
4. What is relationship between gene action and heritability?
5. How gene action is affected by mode of pollination?
6. Why selection is not effective in pure lines?
7. How variation is generated in pure lines?
8. What type of gene action is expected in F1 population?
9. How variation generated in pure lines?
10. What are classifications of genetic variance according to Fisher?
11. What are classifications of genetic variance according to Wright?
12. What are classifications of genetic variance as per Mather?
13. What is genetic constitution of F2 population?
14. What are main features of additive variances?
15. What is additive X dominance Epistasis?

1.7.2 Give an account on following:

1. Phenotype variation
2. Genotype variation
3. Environmental variation
4. R A Fisher
5. Gene action
6. Additive variance
7. Linkage disequilibrium
8. Genetic disequilibrium
9. Additive gene action
10. Dominance gene action
11. Epistatic gene action

1.7.3 Differentiate between the following:

1. Polygenic and Oligogenic trials
2. Phenotypic and genotypic variability
3. Additive and dominance variance
4. Additive and epistatic variance

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1.10. TERMINAL QUESTIONS

Long answer type Questions:

1. Explain briefly genotypic, phenotypic and environmental variation.
2. Give brief account of genetic components of variance.
3. What are the components of genetic variation? Define each.
4. Define gene action. Describe briefly various types of gene action.
5. What is additive gene action? Describe its important features and role in plant breeding.
6. Define non additive gene actions. Give its main features and implication in plant breeding.
7. What is Epistatic gene action? Gives its main features and significance in plant breeding.
8. Describe briefly various methods of estimating gene action in plant breeding populations.
9. Describe in brief the various factor affecting gene actions in plant breeding.
10. Define dominance gene action. Describe briefly its main features and role in plant breeding.
11. Give main difference between additive and non additive gene actions. Also give their merits and demerits.
12. Compare three types of Epistatic gene actions.
13. What are genetic parameters? Outline a method of determining gene actions. How would you utilize the information obtained on gene action in plant breeding?
14. Describe gene action in relation to genetic material and mode of pollination.
15. Define narrow-sense heritability in terms of the components of phenotypic and genetic variation.

UNIT-2 PLANT BREEDING: CONCEPT, OBJECTIVES, ACHIEVEMENTS AND SCOPE

2.1 OBJECTIVES

After reading this unit students will be able-

- to know the concept of plant breeding
- to explain the objectives of the plant breeding
- to know about the achievements of plant breeding
- to understand about the scope of plant breeding

2.2 INTRODUCTION

Plant breeding is a science based on principles of genetics and cytogenetics. It is the science of changing the traits of plants to make them better crops and more nourishing food. Plant breeding has been practiced for thousands of years, since near the beginning of human civilization. Successful commercial plant breeding concern began to be founded from the late 19th century. According to Frankel 1968 the plant breeding is the genetic adjustment of plants to the social cultural, economic and technological aspects of the environment. Darwin's theory of evolution and concept of natural selection, combined with Mendel's work on heredity, became the foundation of plant breeding and selective breeding.

"Plant breeding is the art and science of the genetic improvement of plants". - Fehr, Principles of cultivar Development: Theory and Technique, 1987.

"Plant breeding is the art and science of changing the traits of plants in order to produce desired characteristics". - Sleper and Poehlman, Breeding Field Crops, 1995.

"Plant breeding is the genetic improvement of plants for human benefit."- Bernardo, Breeding for Quantitative traits in plants, 2010.

In the mid-1800s Gregor Mendel outlined the principles of heredity using pea plants and thus provided the necessary frame work for scientific plant breeding. In its most simple form, breeding consists of selecting the best plants in a given field, growing them to full seed and then using that seed to grow further generations. Such selective breeding changes the genetic composition of the plants over time. So, the primary objective of plant breeding is to produce new crop varieties superior to existing types in all characters. Breeding new crops is important for ensuring food security by developing new varieties that are disease resistant, higher yielding, drought tolerant or regionally adapted to different environments and growing conditions. Mendel's work ultimately led to the new science of genetics. Today plant breeding is a specialized technology based on genetics. Modern plant breeding is applied genetics, but its scientific basis is broader, covering physiology, cytology, molecular biology, pathology, systematic, chemistry, entomology, and statistics (biometrics).

The development of newer plant breeding methods did not lead to a complete replacement of the older ones. Depending on the problems plant breeders must be able to choose the tools that enable them to reach their breeding goals in the most efficient and specific way.

2.3 PLANT BREEDING

2.3.1 Concept

Plant breeding is not a new concept. It has been in use since centuries. Plant breeding started with primitive people saving seed to plant in succeeding years. The concept of plant breeding applies genetic principles for producing crops that satisfy requirements of humans. Plant breeding activities can basically be divided into three phases: (a) establishing genetic variation, (b) selection and (c) test phase for selections. The principle method used by man in early days of agriculture was that of selection. Selection is of two types, *viz.,* natural and artificial. Natural selection happens naturally, but selective breeding only occurs when humans intervene. For this reason selective breeding is also called artificial selection.

Natural Selection: Selection which operates in nature without human interference is called natural selection. It favours those plant characters which are necessary for survival (adaptation) of a species. The natural selection is a rule in nature and is the basic cause of evolution. Darwin suggested that in nature, the fittest only survive while rests are wiped out.

Artificial selection or selective breeding: Selection made by human is called artificial selection. It is the process of selecting propagating plants with desirable characteristics and eliminating or "culturing" those with less desirable characteristics. Breeders picks of a few plants of better qualities from mixed populations and tries to propagate them. Selection has resulted in the development of so many varieties of plants, such as Kalyan Sona wheat, Early Leming corn (maize), Res Fife wheat, and several varieties of tobacco**.** Artificial selections are of different type as given below:

1. Pureline selection: Progeny of a self pollinated homozygous plant obtained by selfing is called pureline selection.

2. Mass selection: In this method, individual plants are selected on the basis of phenotype from a mixed population; their seeds are bulked and used to grow the next generation.

3. Progeny selection: A selection procedure in which superior plants are selected from a heterogeneous population on the basis of their progeny performance is called progeny selection.

4. Directional selection: In directional selection, an extreme phenotype is favoured over other phenotypes, causing the allele frequency to shift over time in the direction of that phenotype.

5. Clonal selection: A procedure of selecting superior clones from the mixed populations of asexually propagated crops such as sugarcane, potato, etc. is called clonal selection.

6. Recurrent selection: Recurrent selection refers to reselection generation after generation with intermating of selects to provide for genetic recombination.

7. Stabilizing selection: Stabilizing selection is a type of natural selection in which population stabilizes on a particular trait value and genetic diversity decreases. For example- A plant that is too short may not be able to compete with other plants for sunlight. However, extremely tall plants may be more susceptible to wind damage. Combined, these two selection pressures select to maintain plants of medium height. As a result the number of plants of medium height will increase while the numbers of short and tall plants will decrease.

8. Disruptive selection: Disruptive selection occurs when extreme phenotypes have a fitness advantage over more intermediate phenotypes.

2.3.2 Objectives

The objectives of plant breeding are to improve yield, quality, drought and frost-tolerance, disease-resistance and important characteristics of the crops. Plant breeding can change the genetic potential of seeds, the farmer's raw material, thereby increasing yield, improving quality and enhancing resistance to disease and other natural hazards. Objectives of plant breeding are to develop varieties with better characteristics. Specific objectives would vary greatly depending on the crop under consideration but the chief objective of the plant breeder is to combine in one variety as many of the desirable and economic characters as possible. Some of the main objectives of plant breeding may be summarized as follows:

1. Plant breeding for Disease Resistance: Crop plants are attacked by various diseases and insects, resulting in considerable yield losses. In such situation if the crops are made disease resistant, food production is increased and use of fungicides and bactericides would also be reduced. Genetic resistance is the cheapest and the best method of minimizing such losses. Before breeding, it is important to know the causative organism and the mode of transmission. Some fungal diseases are rust, e.g., red rot of sugarcane, brown rust of wheat and late blight of potato; and some bacterial *viz*. black rot of crucifers and some viral diseases are tobacco mosaic, turnip mosaic, etc. The cultivation of rust resistant wheat, e.g., has been attended with spectacular benefits. Sharbati wheat of Madhya Pradesh is rust resistant. C250, C253 and C228 are the wheat varieties that are highly resistant to yellow rust but not to black rust. Gram varieties G.24 and G.17 have been evolved as a blight resistant cross and are sown in blight affected humid areas. In a number of cases, efforts of geneticists have met with success in this direction but much yet remains to be achieved.

2- Improved Quality: Quality of plant produce is another important objective in plant breeding. The quality of plant determines its suitability for various uses. Quality characters vary from one crop to another. Many of the garden plants are prized for their size, shape, density, bright colour and colour patterns of the flowers, early flowering, late blooming, longer flowering, etc. Similarly fruits are valued for their size, nutritional quality, flavour, vitamin content, amount of juice; crop plants are valued for their nutritional value, yield, lodging and distance, resistance to frost, etc. An excellent example of improvement in quality is long staple cotton. It was produced

by crossing two varieties of Egyptian cotton. Similarly, Sharbati Sonora wheat is produced as a result of mutation in wheat (Sonora 64). This variety is known to have higher protein content than Sonora 64.

3. Increase of Yield: Most of the breeding program aims to increase the yield of grains or fruits per acre. With an increasing population, the production of food needs to increase with it. New varieties of plants can in some cases be developed through plant breeding that generates an increase of yield without relying on an increase in land area. An example of increases production can be seen in the case of wheat, where food production per capita has increased two fold. In 1965 the production was about 12 million tonnes of wheat in a year; in 1970 the production exceeded 20 million tonnes. Increased cultivation of new wheat varieties combined with improved methods of farming has brought about a green revolution in India. Some well known achievements are development of semi-dwarf wheat and rice varieties, production of hybrid and composite varieties of bajra, maize, jowar and noblization of Indian sugarcanes.

4. Varieties suited to particular soils and climates (Cultivation suitability): All crops cannot be cultivated in all types of environmental conditions and soil. It is important that varieties have to be evolved through breeding in order to achieve the desired goal. For example, particular variety may grow in a mountain area, but may not grow well in plains. But by cross breeding; the same crop may be cultivated even in plains. Earlier, the cultivation of groundnut was virtually unknown in Punjab. But a variety of groundnut PG has been evolved and it is now cultivated extensively in Punjab in sandy soils. C145, C117, and C158 are some other new varieties of ground nut.

5. Change in maturity duration: Change in maturity duration permits new crop rotations and often extends the crop area. It permits crop rotation, late planting, double cropping. Development of wheat varieties suitable for late planting has permitted rice-wheat rotation. Maturity has been reduced from 270 days to 120 days in pigeon pea, from 270 days to 170 days in cotton, from 360 days to 270 days in sugarcane.

6. Breeding for tolerance: Crop plants also suffer from abiotic factors as soil salinity, drought, extreme temperatures, frost etc. The major proportion (Ca 70%) of the cropped area is rainfed. The estimates of salt-affected (saline) soils in the country vary from 7 to 20 million hectares, of which about 2.8 million hectares are alkaline soils. Drought and lack of water or nitrogen stress tolerance has become a significant part of agriculture. Breeder has to develop resistant varieties for such environmental conditions which will ensure crops perform under such environmental conditions.

7. Agronomic characters: Improvement in characters like height, branching, tillering capacity, growth habit or trailing habit *etc.*, are often desirable. For example, generally the dwarfness in cereal plant is effective in imparting lodging resistance and better fertilizer response.

8. Non-shattering and non-shedding characters: The shattering of pods in a crop like mung, soya *etc.* and in *Gossypium arboreum* cotton shedding of *kapas* after ball bursting are serious problems. Locule retentive varieties have to be developed in this cotton species

9. Dormancy: In some crops, seeds germinate even before harvesting, if there are rains at the time of maturity, for example, barley, mung *etc*., a period of dormancy in such cases would prevent the loss due to germination. In some other cases, however, it may be desirable to remove dormancy.

2.3.3 Achievements

Over the last 40 years, Indian agriculture has witnessed spectacular advances in both production and productivity of food crops. The successful hybrids show improved characters and an overall increase in yield, growth and size can be seen. Breeding for resistance to the hazards of pests and disease, storms, mineral deficiencies and temperature extremes has had numerous successes. Production is almost doubled in crops, such as cotton, jute, and oil seeds since 1950. Hybridization has added several new qualities to many crops such as wheat, sugarcane, tobacco, *Brassica juncea, B. napus,* dahlias, roses, poppies, *etc*. Systematic wheat research in India started with the establishment of the Indian (Imperial) Agricultural Research Institute in 1905 at Pusa in Bihar. India was the first to release the hybrids of cotton and Pearl millet with remarkable yield potential.

The continuous efforts of many workers like Ramiah in rice, Venkatraman in sugar, Puskarnath in potato, Pal and Ramdhan Singh in wheat, N.G.P Rao in *Sorghum*, Dharampal Singh in oil seeds, Boshi Sen, Paliwal and Dhawan in maize, and Athwal in bajra have put plant breeding on sound footings in India. M. S. Swaminathan is an Indian geneticist, known for his role in Indian Green Revolution, a program under which high-yield varieties of rice and wheat were planted. T.S. Venkatraman, an eminent sugarcane breeder transferred thick stem and high sugar contents from tropical noble cane to North Indian canes. This process is known as nobilization of sugarcane. B.P. Pal, an eminent wheat breeder, developed superior disease resistant N.P varieties of wheat. C.T. Patel is known for famous cotton hybrid in 1970.

Many dwarf and semi dwarf varieties are developed in crop like wheat and rice. Dr. Swaminathan learned of Dr. Borlaug's newly developed Mexican dwarf wheat variety and invited Dr. Borlaug to India. The two scientists worked side by side to develop wheat varieties that would yield higher levels of grain as well as develop stalk structures strong enough to support the increased biomass. Norman E. Borlaug developed semi-dwarf varieties of wheat. Kalyan sona and sonalika are two of the hybrid wheat varieties grown in India. Semi-dwarf wheat varieties were taken from IR-86 (International Rice Research Institute) and Taichung native-I (from Taiwan). Jaya and Ratna are the better-yielding, semi-dwarf rice varieties that were later introduced. In India majority of the wheat varieties grown are semi dwarf, and are resistant to water lodging, responsive to fertilizers doses *etc*. The Indian canes were of

Saccharum barberi, a native of North India and *S. officinarum* of South India. *S. officinarum* had thicker stems and higher sugar content, but it did not grow well in North India due to low winter temperature while *Saccharum barberi* were hardy but poor in yield and sugar content. T.S. Venkatraman and C.A. Barber at sugarcane Breeding Institute, Coimbatore transferred thick stem higher sugar content and other desirable characters from the noble cane to Indian cane which is commonly referred as nobilization of Indian canes.

2.3.4 Scope

There is considerable scope for further modifying the present-day crop species. Population is increasing very rapidly and the food is inadequate. Changing socioeconomic, system deforestation, fast deteriorating soil, desertification, water logging, soil erosion are collectively reducing the agricultural potential of existing land resources. Due to the constraints on land expansion and the increase of food production, it is solely up to the breeders to improve the crop performance and crop yield within the given amount of resources. Higher yields of food plants contribute to a more abundant food supply, a more profitable agriculture, and a lower cost of food products for the consumer. To adequately address these food security challenges, new improved crop varieties need to be developed and reach farmers. Plant breeding has been a crucial tool in increasing production of crops to meet the ever increasing demand for food. Improvements in tolerance to environmental stress, in grain-to straw ratios, and in stand ability, as well as maintenance of required levels of resistance to disease, insect and nematode pests, have been the major genetic causes of higher achieved yields and, will continue to be the foundation for further gains in stability and increase productivity potentials.

For future agriculture to thrive there are necessary changes which must be made in accordance to arising global issues such as, harsh cropping conditions, arable land, food security, *etc*. These global issues are being achieved through the process of plant breeding.

2.4 *SUMMARY*

Plant breeding is the art and science of changing the traits of plants in order to produce desired characteristics for human benefits. Gregor John Mendel, pioneering the principles of heredity, provided the necessary frame work for scientific plant breeding. Selection is an important and essential operation in all breeding program. The selection is of two types, *viz.*, natural and artificial. Natural selection happens naturally, but artificial or selective breeding occurs with human intervention. Artificial selections are of different types, *viz*., pureline selection, mass selection, progeny selection, directional selection, clonal selection, recurrent selection, stabilizing selection and disruptive selection. The objectives of plant breeding are to improve yield, quality, drought and frost-tolerance, disease-resistance, abiotic resistance, *etc*. Indian agriculture has witnessed spectacular advances in both production and productivity of food

crops. Climate change, land degradation, Urbanization are putting pressure on the food supply, to address food security challenges new improved crop varieties need to be developed.

2.5 GLOSSARY

Plant breeding: Plant breeding is the process by which human changes the characteristics of plants over time to make them better crops and more nourishing food. Plant Breeding is the genetic improvement of plants for human benefit.

Heredity: The transmission of genetic characters from parents to offspring

Progeny: Offspring of plants

Phenotype: The phenotype of a plant is a term used to describe observable characteristics, such as height, biomass, leaf shape.

Tillering: Tillering is defined as the process of above ground shoot production by a single plant.

Hybridization: The crossing of two plants or lines of dissimilar genotype is known as hybridization.

2.6 SELF ASSESSMENT QUESTION

2.6.1: Multiple choice questions:

1- A plant breeder decides to develop a disease resistant variety. What should he do first?

(a) Hybridization (b) Mutation

(c) Selection (d) Production of crop

2- Plant breeding is a technique of improving:

(a) Agricultural crops (b) Fodder crops

(c) Fruit varieties (d) All the above

3-Apart from high yield, other main objective of plant breeding is-

(a) Improvement of quality (b) Development of Resistance

(c) Establishment of change in duration (d) All the above

4-Plant breeding is-

(a) an art (b) a science

(c) both a and c (d) None of the above

5-Modern plant breeding started in-

(a) 1850 (b) 1880

(c) 1900 (d) 1930

6- Indian (Imperial) Agricultural Research Institute was established in the year-

(a) 1903 (b) 1905

(c) 1910 (d) 1915

7-Which of the following is not used for crop improvement?
(a) Inbreeding (b) Introduction
(c) Hybridization (d) Mutation

8-Which is the oldest breeding method-
(a) Hybridization (b) Selection
(c) Mutation breeding (d) Introduction

9-The method of selection of plants from population by their phenotype-
(a) Mass selection (b) Pureline selection
(c) Clonal selection (d) Both a & b

10- Sharbati is a variety of –
(a) Wheat (b) Sugarcane
(c) Rice (d) Gram

11-Pureline selection refers to-
(a) heterozygosity and linkage (b) heterozygosity only
(c) homozygosity and self assortment (d) homozygosity only

12-'Kalyan sona' and 'sonalika' are two hybrid varieties of-
(a) sugarcane (b) wheat
(c) rice (d) bajra

13-'Jaya' and 'Ratna' are two high yielding varieties of-
(a) wheat (b) maize
(c) rice (d) none

2.6.1. Answer key: 1-(c), 2-(d), 3-(d), 4-(c), 5-(c), 6-(b), 7-(a), 8-(b), 9-(a), 10-(a), 11-(d), 12-(b), 13-(c)

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2.9 TERMINAL QUESTIONS

1- Define Plant breeding. Discuss about its concept.
2- Describe in detail about the objectives of plant breeding.
3- Write a brief note on the achievements on plant breeding.
4- Give a brief note on the scope of plant breeding.
5- Differentiate between-
(a) Mass selection and Pureline selection.
(b) Natural selection and Artificial selection.

UNIT-3: PLANT BREEDING METHODS

Contents

3.1 OBJECTIVES

After reading this unit students will be able to know-

- Study of different methods of plant breeding for self- pollinated and cross- pollinated species.
- Study of merits and demerits of different breeding techniques.
- Importance of different plant breeding methods.

3.2 INTRODUCTION

The main object of plant breeding is to produce the new and higher vigor, disease resistant crop varieties superior in all aspects as compared to the existing types. Plant breeding is the purposeful manipulation of plant species in order to create desired genotypes and phenotypes for specific purposes. To achieve these objectives different crop methods are applied by plant breeders and agronomists. Plant breeding has been practiced for thousands of years, since near the beginning of human civilization. It is practiced universally by individuals such as gardener farmers and by professional plant breeders employed by organizations such as government institutions, universities, crop specific industry associations or research centres established specially for plant improvements. The process of plant breeding is assumed about 7,000 years ago with the beginning of human civilization. Domestication of wild species under human management is use as source of food. Since beginning the human beings used nomadic practices and it helped the movement of cultivated plant species. Various approaches viz. selection, hybridization, mutation etc. that are used for genetic improvement of crop plants are referred to as plant breeding methods or plant breeding procedures or plant breeding techniques. The choice of breeding methods mainly depends on the mode of pollination, mode of reproduction, gene action and breeding objectives of crop species.

Plant breeding is the science of changing traits of plants in order to produce desired characteristics. Various breeding procedures that are more commonly used for the genetic improvement of various crop plants are known as general breeding methods. Such breeding methods include introduction (pureline selection, mass selection, progeny selection, etc.) hybridization (pedigree, bulk and back cross methods), heterosis breeding, synthetic and composite breeding. Similarly, there are some other breeding methods, rarely used for improvement of crop plants are referred to as special breeding methods, including- mutation breeding, polyploidy breeding, wide crossing or distant hybridization and biotechnology.

3.3 PLANT BREEDING METHODS

The main object of plant breeding is to produce the new and high vigor, disease resistant crop varieties superior in all aspects as compared to the existing types. To achieve these objects different crop improvement are mentioned below. All these methods have been dealt in this unit only in brief while a detailed account of each has been given in the text separately.

- Selection
- Hybridization
- Plant Introduction and acclimatization
- Mutation Breeding

3.3.1. Selection

Plant improvement is an ancient art or science started with the primitive man changing his mode of life from a nomad to agriculturists and might have the first crop from nature in wild form or he had never sown the seeds to ensure the first crop (**Fig. 3.1**). So to obtain his first crop he unconsciously might have practiced the selection by selecting the best one. It is therefore, the oldest breeding method and is the basis of all crop improvements.

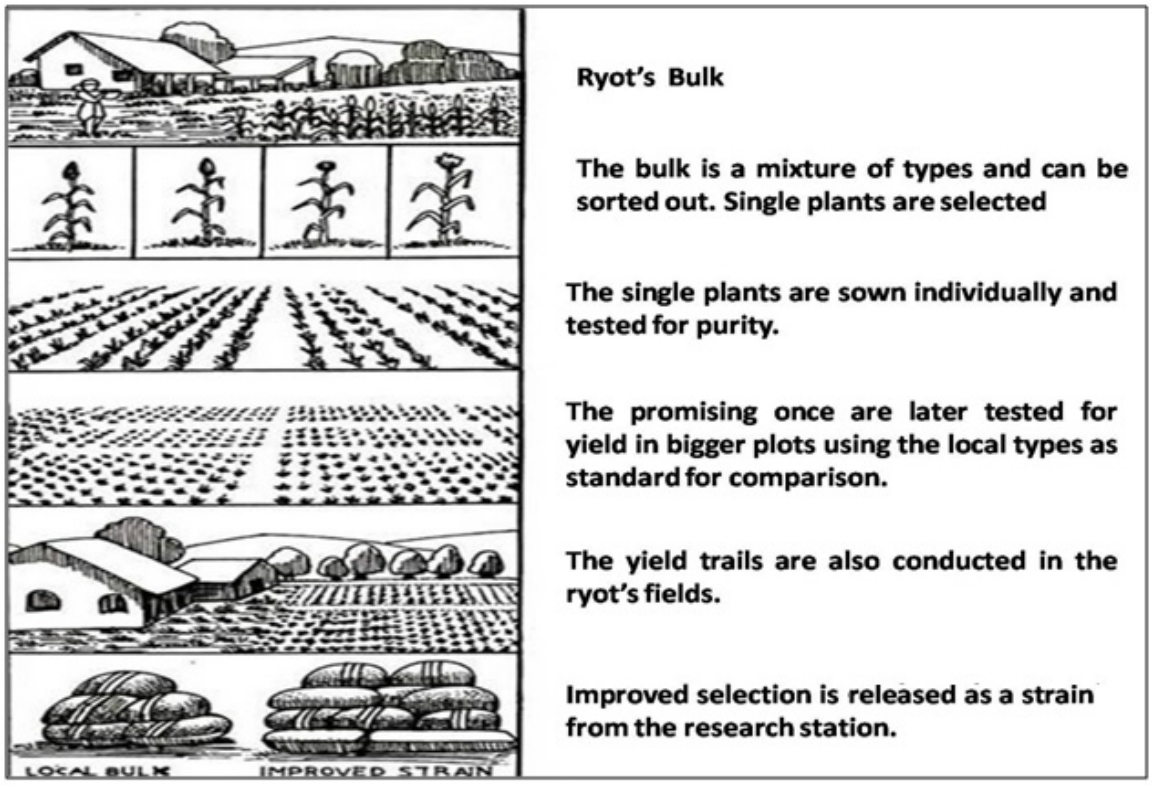

Fig.3.1: Schematic presentation of crop improvement methods

It is the most common and popular method of crop improvement among the cultivators even today divided in to two categories.

a. Natural selection and
b. Artificial selection

a. Natural Selection: Natural selection as apparent from its name is a rule in the nature that nature selects the best and fittest for future and, discarded the inferior through evolution.

According to natural law the fittest can survive and rest wipe out. This has given the cultivated crop and forms ecotypes in plants. Based on climatic or regional specialties forms climatic or regional races are the basis of artificial selection and hybridization.

Through this process local varieties of crops are produced. Sometimes many variations arise between species and sub- species due to selection pressure. It remains always operating in nature and is one of the natural factors resulted variations in the already existing varieties of crop in the nature. Besides natural selection, crop breeders and agriculturists also practice artificial methods for selection and known as artificial selection operating in nature and is one of the natural factors resulted variations in the already existing varieties of crop in the nature.

b. Artificial Selection: The cultivators and plant breeders select special types of plants from the mixed population of crops for their own advantages. This type of selection is known as artificial selection (**Fig. 3.2**) and can be defined as "artificial selection is to choose certain individual plants for the purpose of having better crop from a mixed population where the individuals differ in characters".

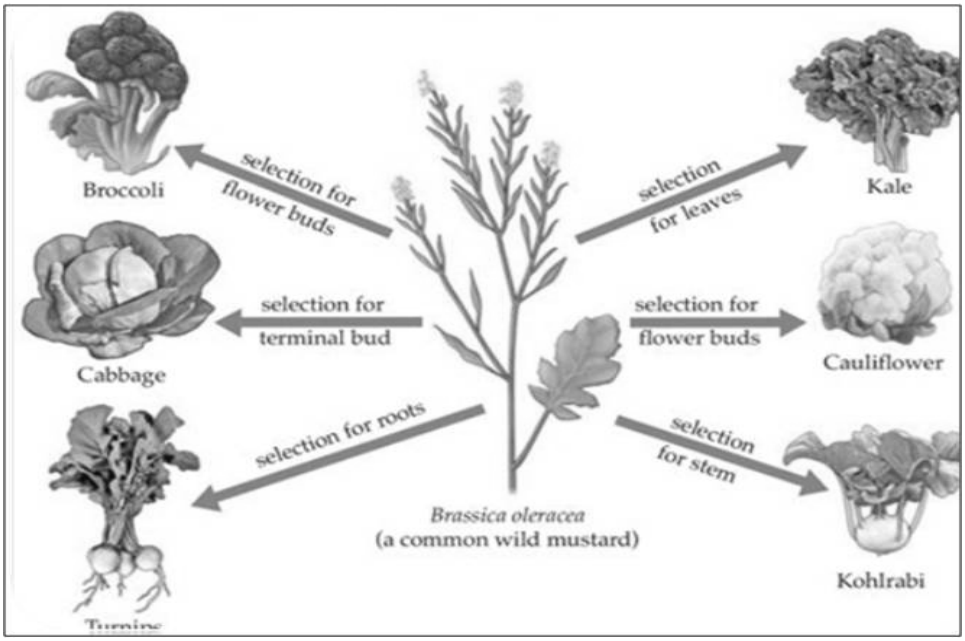

Fig. 3.2: Artificial cultivation of mustard

Artificial selection is further categorized into two sub- divisions on the basis of nature of crop selection among a mixed population. It may be as below-

i. Mass selection
ii. Pure- line selection and
iii. Clonal selection

In nature there is continuous selection by natural forces like temperature, soil, weather, pests, diseases, etc. The genotype which is more suited to a given environmental conditions leave behind others which are less adaptive in nature. The procedure selection involves the retention of superior phenotypic plants from mixed population. This can be done in different ways in different crops.

i. Mass Selection: When a large number of plants of similar phenotypes are selected and their seeds are mixed together to constitute a new variety, the procedure is called mass selection. The population obtained from the selected plants would be more uniform than the original or existing population. It is followed in both self- defective and cross- pollinated crops. Collecting and selecting the best, healthy and more vigorous plants or seeds from the mixed population of crop. All the selected plants are thrashed together and obtained a mixture of seeds. This mixture of seeds is a mass, so this method is known as mass selection. The mixture of seeds is sown to raise a new crop. Again in the next year selection is made similarly by adopting the selection of best ones. This practice of selection is continued till the plants show uniformity in the desired characters and constitute a new variety. Mass selection cannot be done if the population is already uniform. Thus mass selection is based upon the presence of variability in the population. As a general rule it is known that greater the variability better are the results of selection. The new varieties developed by mass selection is more or less pure or similar in external features because the plants are selected on the basis of external or phenotypical characters which may be easily observed and used in identifications.

Thus by using this method a number of varieties had been obtained and some of the outstanding strains till recently were the products of this selection. The purpose of mass selection procedure in case of self- pollinated crops has following objectives:

1. Improvement of local varieties
2. Purification of existing pure line varieties and
3. Production of new varieties from heterogeneous local land races.

Merits and Demerits:

1**.** In this method since a large number of plants are selected hence the variety is more stable in performance over different environment as it is more adopted than a single pureline selection. Thus the varieties developed through mass selection are more widely accepted than pureline.
2. This method is less labor consuming extensive and prolonged field trials are not necessary and hence reduces the time and cost needed for developing a new variety.
3. Mass selection retains considerable genetic variability and another mass selection after few years does not improve the variety.
4. This method can be applied to cross pollinated crops.

5. This method utilizes only the variability which already exists in the population; improvement is done only through selection. So the limitation is that it can not generate new genetic variability.

Achievements: Mass selection is effective when the population has the following characters:

1. The characters should be highly heritable in nature.
2. High genetic variability exists for different traits.
3. The crop is strictly grown under low population density.

ii. Pure-line Selection: In contrast to mass selection, pure line selection comprises of selecting a large number of plants from a population of self- pollinated crops and harvested individually. Their individual progenies are cultivated separately and evaluated. The best progeny is released as a pureline variety. Thus a pureline consists of a progeny of a single self-fertilized homozygous plant and is used for developing a variety (**Fig. 3.3**). This method of production of a variety from the pure line is known as pure line selection. It is used in the mixed population of self- pollinated crops. In this method the progeny of single individual plants tested separately.

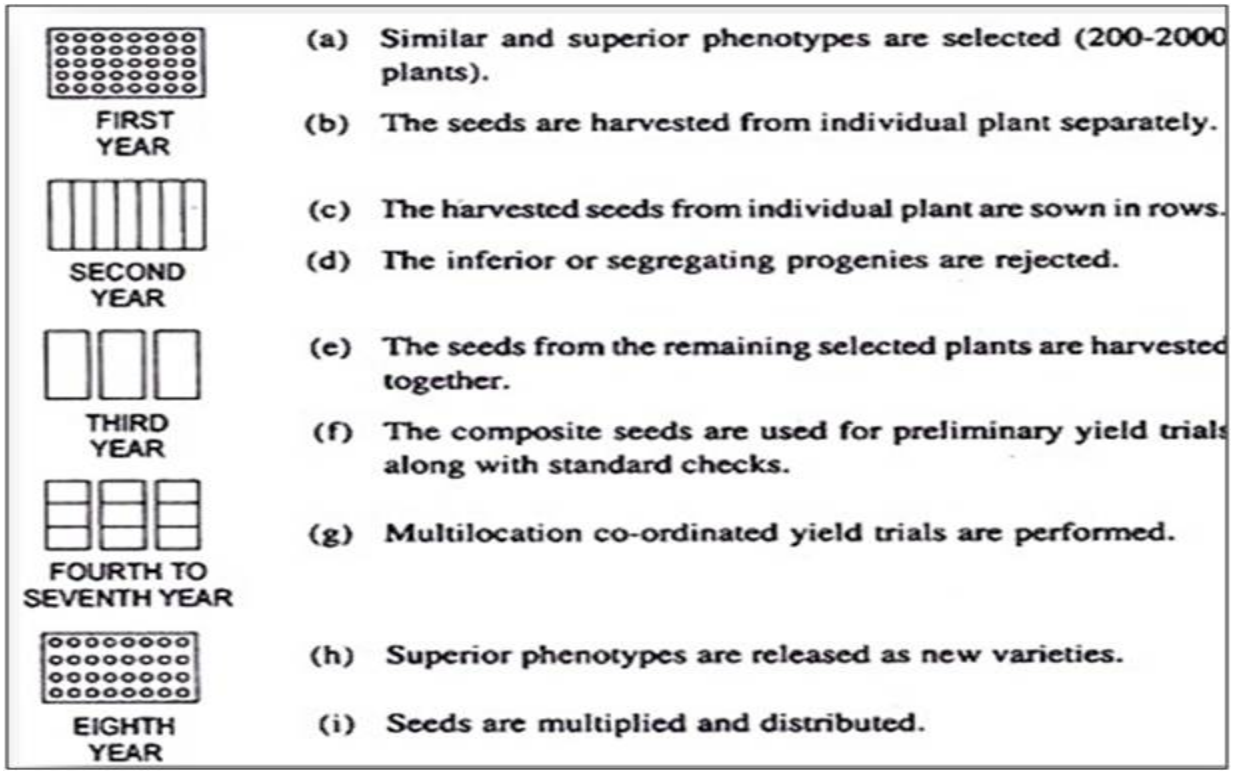

Fig. 3.3: Different steps applied in pure-line selection

Usually large number of single plants are selected and compared to save the single most valuable progeny as a new variety for future. Therefore, this method is not materially different from mass

selection except that in this method fewer plants are selected and each selected plant is tested separately. Thus the variety developed through pure- line selection is genetically pure and more durable than the previous one. This method is not only practiced for self- pollinated crops but also in cross- pollinated crops during hybridization for production of pure- lines to serve as the parents in crossing. As stated previously the pure- line is a progeny of a single homozygous self-pollinated plant. In self- pollinated crops pureline selection method has several applications as below:

a. It is a favourable method of improvement of local varieties which have considerable genetic variability.
b. Pure- line selection can also be done for introduced varieties.

iii. Clonal Selection: Clonal selection is generally practiced in vegetatively propagated crops such as potato, sweat potato, banana, sugarcane, mango, citrus, onion, etc. In this case a group of plants obtained vegetatively from a single plant is known as a clone and the variety developed from the clones is referred as clonal selection (**Fig. 3.4**). After that on the basis of phenotypic characters the superior clones are selected.

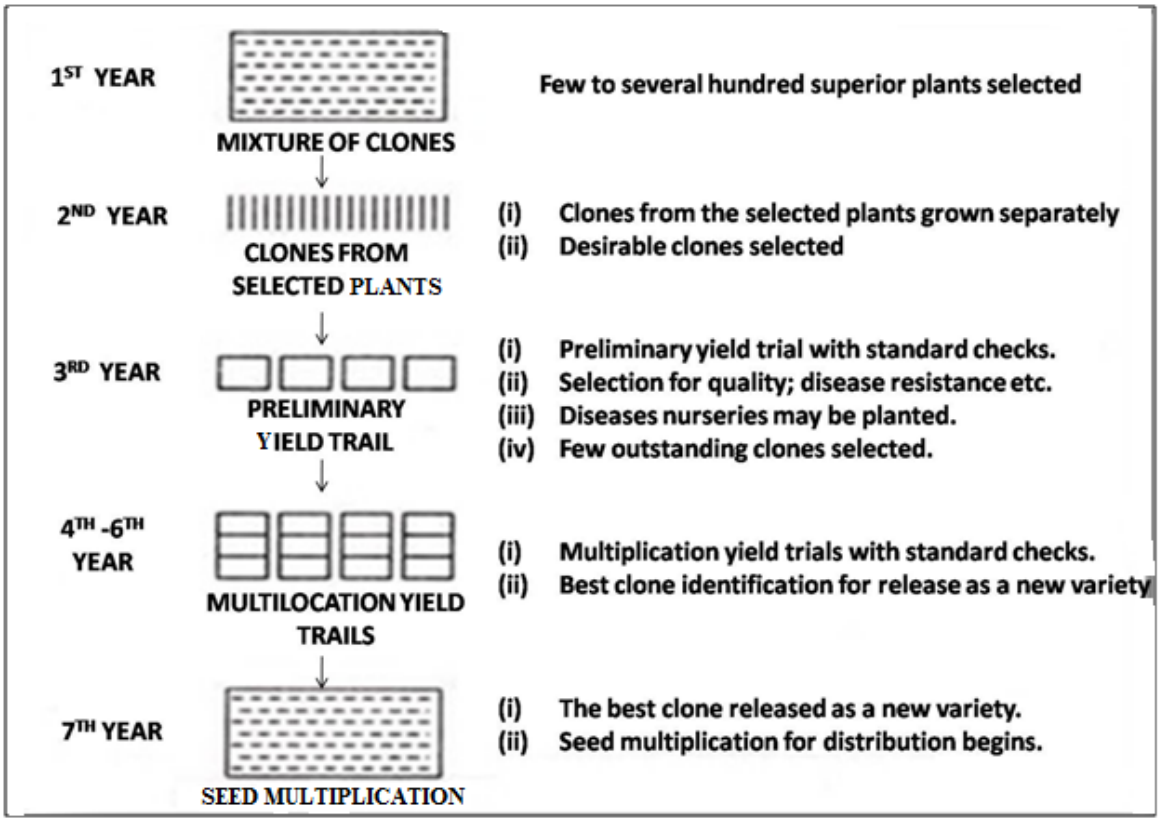

Fig. 3.4: Steps applied in clonal selection

Bud selection is also a form of clonal selection where bud is the unit of selection. This method is applied in the improvement of fruit crops such as oranges and mangoes, etc.

The fundamental basis of this selection is always among the clones and never within a clone because all the individuals of a clone have the same genetical constitution. These selected clones are multiplied vegetatively and compared with normal variety. The best ones are selected, multiplied and tried at different stations or areas continuously for three years. The best clones are given name and released as improved variety. Clonal selection is similar to pureline selection in vegetatively propagated crops, since vegetatively propagated pure- lines are the basis of improvement.

3.3.2 Hybridization

i. Pedigree Method: Pedigree method refers to record of the ancestry of an individual selected plant pedigree breeding and, is a genetic improvement of self- pollinated species in which superior genotypes are selected from segregating generations. Proper records of the ancestry of selected plants are maintained in each generation. It is a selection method which is used in segregating population of self- pollinated species keeping proper records of plants and progeny selected in each generation hence known as pedigree breeding. This method is widely used for the development of various varieties in self- pollinated crops. Pedigree method of crop cultivation was first described by **H. H. Lowe** in **1927**.

Application: This method is widely used for the improvement of self- pollinated species. It is largely used when both the parents involved in the hybridization have good agronomic characters or are well adopted. However, this method is also used in cross- pollinated species. Mostly it is commonly used for the improvement of polygenic traits than oliogenic characters. In self-pollinated species it is used for development of new varieties, while in cross- pollinated species for development of inbred lines. Thus the main difference between these two type species (self and cross pollinated) is that in the self- pollinated species inbreeding occurs naturally while in cross- pollinated species inbreeding is done manually.

Maintenance of Pedigree Method: In pedigree method proper record of the ancestry of each selected plant or plant progeny is maintained for all generations of different selection. Important characters of each selected plant and plant progeny are recorded separately every time.

Selection of Pedigree species: In this method only human selection or artificial selection is used where natural selection is allowed to operate only in the modified form of pedigree breeding known as mass pedigree method.

Time Taken: This is a long time taking method of plant breeding. Development of new crop cultivar by this method generally requires 14 - 15 years.

Genetic Constitution: The variety developed by this method is homozygous and heterozygous, because it is a progeny of a single homozygous plant.

Breeding procedure for pedigree method: As mentioned earlier it is a time consuming procedure and is outlined below (**Fig. 3.5**):

1. First parents are selected keeping in view the requirements or objectives.
2. Cross is made between selected parents.
3. Using wide spacing F_1 Parents is grown.
4. For various desired characters the dominance behavior is recorded.
5. In F_2 generation also the material is grown using wide spacing.
6. In F_2 the individual plant selection is practiced.
7. The progeny of each selected plant is grown separately which constitutes F_3 generation.
8. In F_3 and F_4 generations, selection is practiced within and between the families.
9. Between progeny selection is practiced from F_5 to F_8 and superior progeny are identified and isolated in F_8 progeny.

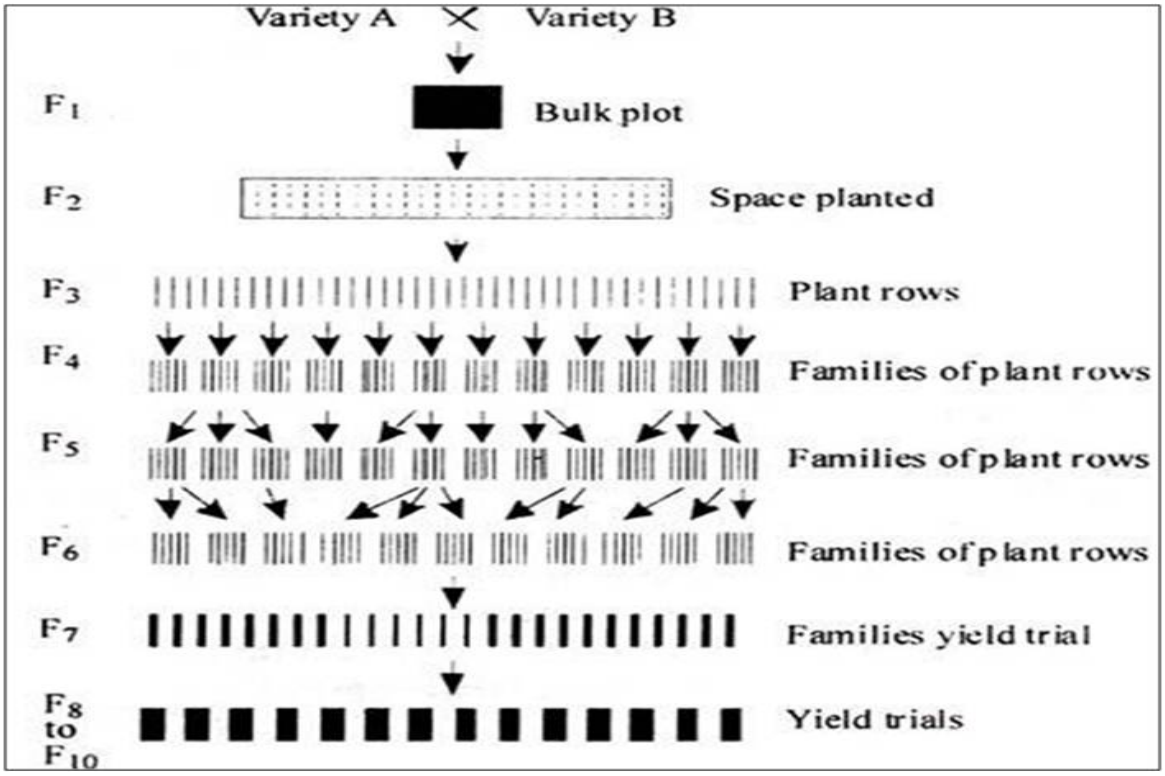

Fig. 3.5: Different steps involved in pedigree method

These progeny constitute strains. These strains are evaluated in replicated multi location trials for a period of 3 - 5 years. The final strain is released as a variety based on superior performance. Thus release of a new variety by this method takes 14 -15 years.

Achievements: Pedigree method has been extensively used for developing improved varieties in several self- pollinated crops like rice, wheat, pulses, maize, barley, cotton and several vegetable crops.

Merits of pedigree method: The pedigree selection is based on genotypic value rather than phenotypic value because the breeding value of selected plants is ascertained by progeny. This method provides information about the mode of inheritance of various quantitative characters which is not possible by other breeding method. This method takes 14 - 15 years to release as new variety whereas bulk method takes much longer time around 15 -16 years.

Demerits:

1. In this method since large numbers of progeny are rejected, since, there are chances of elimination of some valuable material also.

2. Due to continuous selection of individual progenies the selected material becomes so large that hardly of the same becomes very difficult.

3. For proper information records have to be maintained for all the selected plant and progeny which take lots of valuable time of a breeder.

ii. Back Cross Method: Backcross method was first proposed by Harlan and Pope (1922), applied for the breeding of small grains. It is totally different from bulk method and pedigree method. This method is employed for both types of crops i.e. self and cross pollinated where varieties are different in one or two aspects especially for transferring a single character like resistance to disease, frost, drought and early maturing one from an undesirable (less important) variety to a good commercial variety. Hence in back cross there are two types of crops- one is the desirable variety called "recurrent" or "recipient parents' and another undesirable variety called "donor parents", possessing a character which lacks in the good parents. The first one variety is crossed with the second one to obtain F_1 generation. The F_1 generation is crossed with the recurrent parents and therefore this is known as ***back cross method***. The main aim of back cross is to recover the genotype of recurrent parent with disease resistant character. The first progeny of such cross is known as BC_1 in spite of F_1 which consist and containing the good characters from donor parent and again back crossed to the recurrent parent. To achieve the plants with desired characters back crossing is continued usually up to BC_5 or BC_6. The plants possessing desired characters of non-recurrent parents are primarily selected in each back cross generation.

At the end of BC_6, the selected desired plants are self- pollinated to make the population homozygous in all combined characters. Thus the plants obtained, must very closely match or similar with the original recurrent parents.

Example: As for example one variety is very good in all characters represents A, but disease susceptible while another variety is disease resistance but very poor in all other characters,

represents as B. Thus to get a disease resistance, it is needed to transfer the disease resistance from B to A without affecting good qualities. Here B is donor or non- recurring parent while A is recipient or recurrent parent.

Procedure: 1. Select the plants of A and B variety, where A represents as female and B as male parent. Both parents are crossed to obtain F_1 generation. Plants possessing desired characters of A with disease resistance of B are retained and rest wiped out. Note the results and collect them.

2. This F_1 (A x B) is back crossed with A, the mother parent i.e. F_1 (A x B) as male and A variety as female to get F_1 back cross plants or BC_1- (back cross first generation). To check disease resistance property inject artificially with disease spores. Plants possessing desired characters of A and B are selected.

3. Selected BC_1 plants are again back crossed with A to raise BC_2 and selection is further made similarly and in step 2.

4. This process of backcrossing to A and select the back cross progenies till a desired type having good qualities of A and disease resistance of B is obtained. Thus transfer of some valuable genes of one species as in case B variety into the genotype of another species as here is A variety by crossing is also known as “Introgressive hybridization”.

5. Finally the selected plants are self- pollinated to make them homozygous for disease resistance.

6. The selected self- crossed lines can be crossed together to restore hybrid vigor (**Fig. 3.6**).

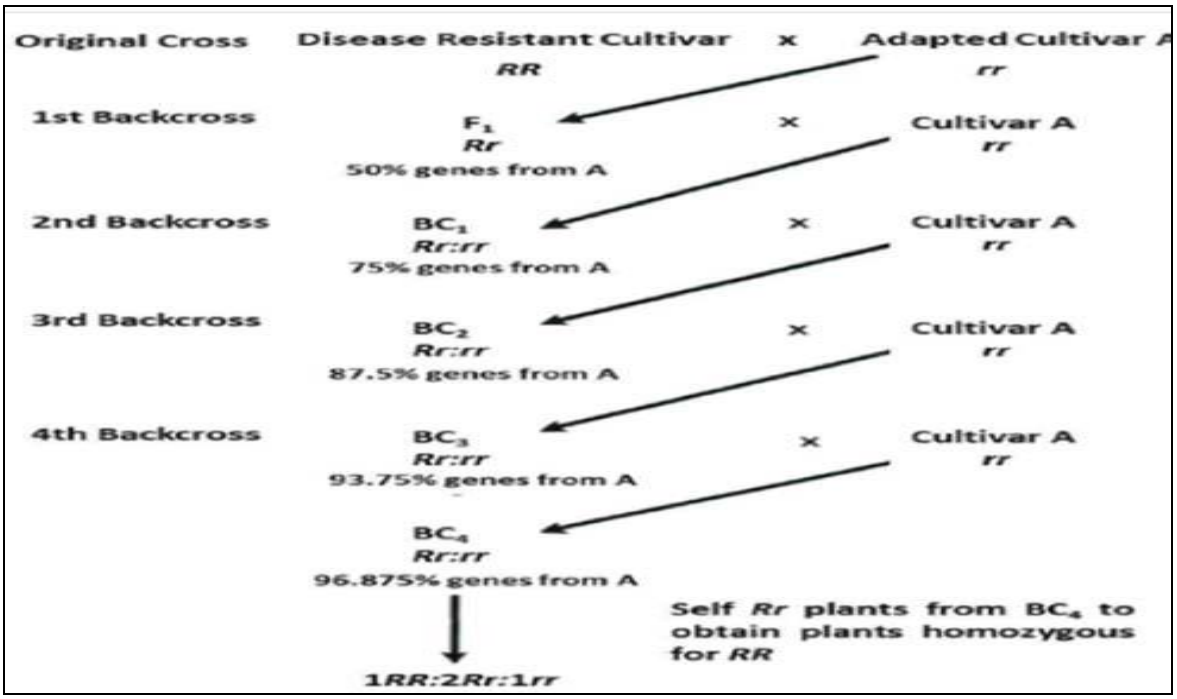

Fig. 3.6: Different steps involved in back cross method of plant breeding

Finally when two different characters of a donor variety have to be transferred, it is good to transfer each character in a separate organism. Back cross method is generally used for transferring disease resistance from one variety to another variety and disease resistance may be dependent on a dominant or recessive character. It is relatively easy to transfer with a back cross in each generation if it is determined by dominant gene but when it is due to recessive gene, each back cross is grown to F_2 to permit the identification of homozygous recessive genotype.

Another way is to back cross twice or thrice in successive generations and to grow yield product of second or third back cross to find homozygous plants for recessive gene under transfer.

Advantages

1. It requires small number of plants and is rapid compared to bulk method and pedigree method.
2. It is repeatable.
3. It is predictable.
4. It is independent of environment.
5. It is little record keeping.
8. It is applicable for both dominant and recessive gene transfer.

Disadvantages

1. It is a lengthy process, by the time we can transfer a gene to have a variety that variety may have been replaced by another superior variety.
2. If undesirable genes are closely linked to desired genes the undesirable genes are probably transferred along with the desired one to new variety.
3. It is ineffective for traits with low heritability.
4. New variety cannot be superior to recurrent parents except for the character transferred.

iii. Bulk Method: In this method the F_3 plants are not maintained separately. Again the suitable plants are selected, collected and bulked together in F_3.

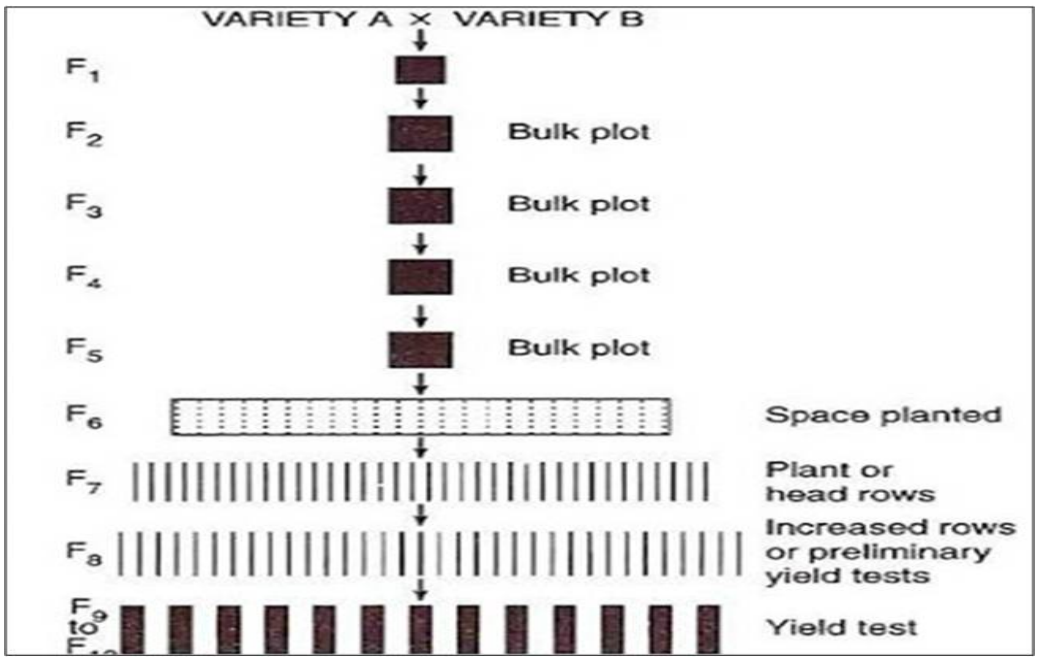

Fig. 3.7: Different steps involved in bulk method of plant breeding

This bulking is continued till the sixth generations. In sixth or F_6 the desired individual are selected and harvested separately. Then the produce of each plant is separated and carried out under comparative trials. The best ones are selected again and released as new variety (**Fig. 3.7**). It is a method which can handle segregating generations in which F_2 and subsequent generations are harvested in bulk to grow the next generation. At the end of bulking period, individual plant selection and evaluation is carried out in the similar fashion as in pedigree method.

Advantages

1. It is simple and inexpensive method.
2. It is little record keeping.
3. It is easy to handle populations as bulk harvest is done.
4. Natural selection is effective.

Disadvantages

1. It requires more time duration to develop a new variety.
2. It is not a suitable method for green houses.
3. Not much scope for skills.
4. No recombination occurrence among superior lines.
5. Natural selection may also work against desirable traits.

iv. Multiple or Composite Cross: Several selected pure lines can be combined using this method. Single crosses between these lines are performed for instance if we have eight pure lines

viz., A, B, C, D, E, F, G and H, then the single crosses may be made as A x B, C x D, E x F and G x H and so on.

F_1 of these single crosses are then combined into double crosses as (A x B) x (C x D) and (E x F) x (G x H) and finally get F_1 of these double crosses, again these double crosses are crossed with each other to produce hybrids [(A x B) x (C x D)] x [(E x F) x (G x H)]. This is known as multiple cross. After that further breeding in these hybrids is carried out according to either bulk or pedigree method as described earlier. In self- pollinated crops multiple cross is used when more than three or four monogenic characters are scattered in three or four different varieties.

v. Cross Pollinated Crops- For cross pollinated crops, the inbreds are combined in any one of the following crosses and released as improved strains:

- Single cross
- Three -way cross
- Double cross
- Top cross or Inbred cross and
- Synthetic cross

Single cross: (A x B) - When a cross is made between two inbred then it is known as single cross such as (A x B) or (C x D). This was proposed by Shull (1909). The seeds of hybrid or its first generation products are distributed to farmers for raising the crop.

Procedure: To obtain a single cross two rows of female line to one line of male planted alternatively so that two third of the field can produce hybrid seeds for sale. The number of possible different single crosses produced from various inbred can be calculated by the following simple formula-

Number of single crosses = n (n - 1) / 2 (Where n = no. of inbreds)

Example- If 4 inbreds A, B, C, and D are involved, the no. of single crosses produced will be

N= 4 so number of single crosses = 4 x (4 - 1) / 2 = 4 x 3/ 2 = 12/2 = 6

A x B, A x C, A x D,
B x C, B x D,
C x D (Total = 6).
Likewise this if inbred are six then the single crosses will be - 6 x 5 /2 = 30 /2 = 15.

A x B, A x C, A x D, A x E, A x F
B x C, B x D, B x E, B x F
C x D, C x E, C x F
D x E, D x F,
E x F (Total = 15).

The significance point of single crosses is that it gives the maximum possible degree of hybrid vigor. In USA these crosses are generally used for production of sweet corn for canning or freezing, while their kernels are very small in size and seeds are poorly developed caused high cost of seed production on commercial scale. Commercially these crosses are not preferred by farmers/ breeders but have significant importance primarily as foundation hybrids for double and three way crosses and also used for prediction of the performance of double crosses.

Double Crosses: (A x **B)** x **(C** x **D):** A cross in which each parent is the product of a single cross; it can be represented as AB x CD, where A, B, C and D are inbred lines. This type of cross is known as double cross. In 1918, Jones used this for the first time. It is not necessary that the inbred involved in one single cross must not be included in the other single cross of the double cross parents. For best results it is also suggested that in the single crosses similar or closely related inbred may be combined while different or distantly related inbred combine in the double crosses.

Procedure: To obtain double cross alternate planting of two single cross plants in an isolated plot and data selling of the single cross used as female parent. Usually the ratio of the female to male rows has been 4:1 so that 80% of the field produced seeds for sale. The formula to calculate the possible double crosses is as follows-

No. of double crosses = n (n-1) (n-2) (n-3)/8 (Where n= Number of inbred involved)

If there are 4 inbreds i.e., A, B, C and D involved in double cross

The possible double crosses will be= 4 (4 -1) (4-2) (4-3) / 8

= 4 x 3 x 2 x 1 / 8= 24 /8 = 3.

Or [A x B) x (C x D)], [(A x C) x (B x D)], [(A x D) x (B x C)] = 3 double crosses.

Double crosses are widely used in commercial hybrids. The best example is the commercially used maize hybrid seeds which are the results of double crosses. These double hybrid gives very high yield in small land without increasing the cost of production. They can fulfill the demand of farmers at a price which is affordable and payable to farmers being produced in large quantities. However, comparatively to single crosses, double crosses have been more variables and the variability has often given them wider adaptability.

Three Way Cross: (A x **B)** x **C:** when a cross between a single cross and inbred involved in which single cross used as female and inbred used as male or three inbreds (A, B and C) involved. In this the inbred and the single cross are planted in the same way as the inbred in the single cross. However, the use of vigorous hybrid of first generation as female in order to minimize the yield of hybrid seed as well as to obtain seeds of normal kernel size is the advantage of three way cross. While it is difficult to obtain high pollen production from the

inbred male parent is the only disadvantage of this cross. This cross gives intermediary results comparatively to single and double crosses.

Top Cross or Inbred Variety Cross (A x **Variety):** This cross performed between an open pollinated variety and inbred line. In this a variety is preferably used as female, while the inbred may be used as male parent. The top crosses are used mostly for testing the combining ability of inbred and not for commercial hybrid seed production.

Synthetic Cross: A variety which is developed by inter crossing in all possible combinations a number of inbred lines with good general combining ability and mixing their seeds (seeds of F_1 generation) in equal quantity is referred to as synthetic variety. It is relevant to cross pollinated species.

The basic concept in the development of synthetic variety is exploitation of heterosis. In the development of synthetic variety heterosis is partially exploited because some degree of inbreeding takes place due to open pollination in later generations.

3.3.3 Introduction and Acclimatization

Plant introduction and Acclimation (**Fig. 3.8**) is the easiest and most rapid method of crop improvement in which the acclimatization follows the introduction and both the processes go by side. According to Chaudhary “Plant introduction is the process of introducing plants from their growing locality to a new locality”, or we can say that plants are transferred from one place to another having different climatic conditions. However, Acclimatization is the adjustment of an individual plant or plant population in a changed climate for a number of generations. Introduction as a method of plant breeding involves the transfer of genotype or population from one environment of production to another. The plant material so introduced may reproduce its performance as such or may get adapted. This is referred as acclimatization.

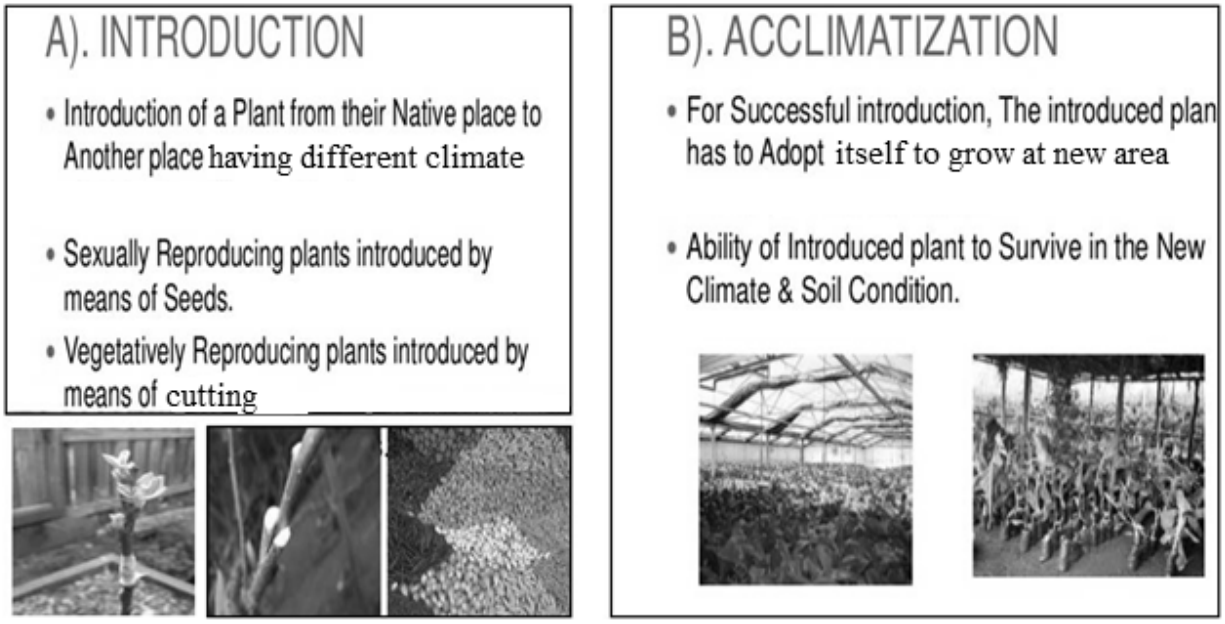

Fig. 3.8: Introduction and Acclimatization method of plant breeding

Growing plants to a new locality with different climate is termed as plant introduction and their adjustment under such changed climate of the new locality is known as acclimatization. Introduction of new crops as well as new varieties of crops may be introduced either in the form of seeds or cuttings. In sexually propagated crops the seeds are imported while in vegetatively propagated crops the cuttings are imported. The crops are generally introduced since they have greater frequency of gene recombinations owing to the frequent cross - pollinations and some of the combinations may be more favorably adopted in the new environment. Introduction of crops may be done into a locality either from outside the country or from different regions within the country as per need and suitability. In fact, plant introduction within the country are very convenient but introduction from another country followed a definite procedure as below:

1. The desired material is demanded from the concerned authority or agency of the foreign country through the plant introduction organization of the country.

2. After proper packing the material is sent by sea.

3. Before entering the country, this material is inspected at sea port by the plant protection authorities and,

4. After certified fit according to quarantine rules, is permitted to enter in the country.

5. In the country it is handed over to the concerned institutions or workers.

6. Then it is grown under local climatic conditions and tested for acclimatization and presence of desired characters.

7. The material if proved fit for both, it is either utilized as such after selection or utilized as a material in hybridization for transferring the desired characters into the local varieties.

3.3.4 Mutation Breeding

Sudden heritable variations in the plants other than those due to Mendelian segregation are known as mutation. These changes are due to rearrangement of genes or gene mutation or point mutation (**Fig. 3.9**). When change occurs in chromosomes size and structure, it is termed as chromosomal mutation, while change in the chromosome number is referred as polyploidy. However, when changes occur in the plant body, it is known as somatic mutation. All these types involve hereditary changes or changes in genotypes of the plants and give rise to inexhaustible variations which besides providing the new material for evolution are the basis of selection and production of new crop varieties in the plant breeding.

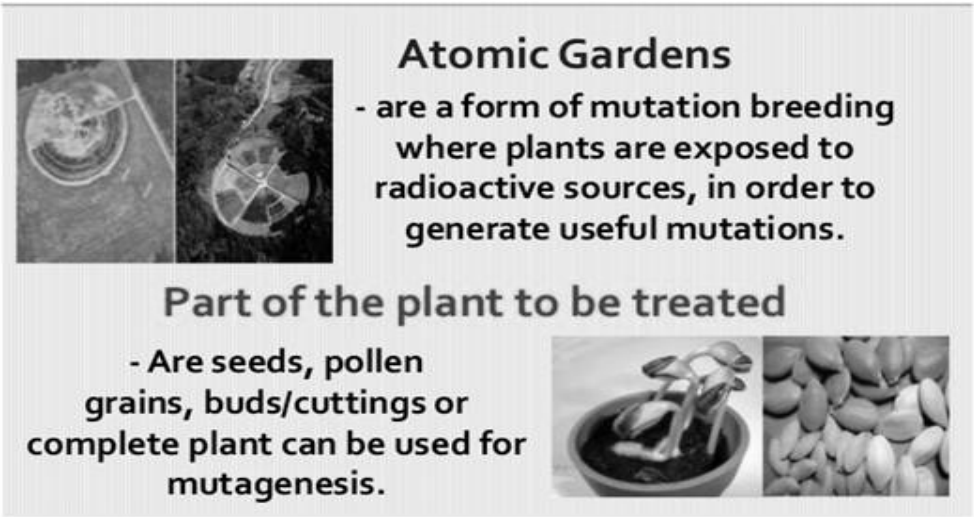

Fig. 3.9: Different aspects of Mutation breeding

At present various types of mutagenic substances/ mutagenic facilities are available for breeding which gives persistent results. There are a number of mutagenic substances used for plant breeding as follows:

A. Physical Mutagens

i. Radiation: The movement of energy either in the form of particles or wave form which is giving off by radioactive isotopes through a space is considered as radiation. It includes Alpha rays, Beta rays, X- rays, Gamma rays and Neutrons. They can transfer their kinetic energy to any matter through which they pass is known as particulate or corpuscular radiation (the radiation in the form of high energy). This radiation in the form of high energy short wave causes magnetic and electric disturbance and affects the internal structure of matter and are known as electromagnetic radiation. The treatment is called “Irradiation”.

Alpha rays: These are radioactive rays made up of two protons and two neutrons with positive charge. They are emitted chiefly by the isotopes of heavier elements and when pass through a matter create strong ionization. They have very little penetrating power in living tissue because being positively charged they are slowed down readily by the negative charges in matter. Alpha rays usually cause the chromosome aberration.

Beta rays: Beta rays are also radioactive rays with negative charge. They are high speed electrons emitted from the nucleus of an unstable atom. They have very less ionizing power but greater penetrating power than α rays. Being negatively charged, they are slowed down by positive charges. They cause both chromosomal aberration and gene mutation. P^{33} and S^{35} are used as the source of these rays and are available in the form of chemicals out of which solution is prepared and desirable treatment is given.

X- rays: These are source of electromagnetic radiations having wavelength much shorter than those from visible light. They are produced in abroad ranging energies. Soft x- rays of longer wave length (10 to 10 A^0) are less penetrating but more densely ionizing than the shorter wave length (0.1 to 0.05A^0) hard x- rays. Average wave length of x- ray from x- ray machine is generally 0.5 A^0. They have relatively sparsely ionizing electrons i.e. effects are due to sparsely ionizing electrons.. X-rays transfer their energy to the atom of tissue through which they pass, which cause as ejection of planetary electrons due to ionization and excitation of atoms or molecules and consequently giving rise to chromosomal and gene mutations. The source of x-ray treatment is x- ray machines installed in rooms.

Gamma rays: Gamma rays are electromagnetic radiations similar to x- rays in their physical characteristics and actions on the organisms. They are therefore, natural x- rays but of very short wave length by virtue of which they are more penetrating. Gamma rays are of same nature as is light but have shorter wave length and therefore, contain higher energy. Most of the Gamma rays have wave length of less than 0.01A^0 as compared to 4000 to 7000 A^0 of high and 0.5A^0 of x-rays. Gamma rays ionizing effects are also due to high electrons ejecting from the atoms of tissue through which they pass.

Neutrons- They are electrically neutral particles and their biological effects are due to the densely ionizing protons which may be fast as well as slow. The neutrons may be highly penetrating because due to lack of any electric charge they are not slowed by charged particles of matter and thus tend to move in a straight line until they have a collision. The sources of treatments are nuclear reactors and generators available with Atomic Energy Department of Government in different parts of the country.

ii. Ionization- During radiation the high energy radiations both particulate and electromagnetic pass through the matter and can cause ionization. It includes ultraviolet rays and visible light. The process by which production of ions (electrically charged atoms or molecules) takes place is known as ionization. When a fast moving charged particle passes through a matter and pulls an

electron out of the orbit of an atom then the particulate radiation can produce ionization. This atom then becomes an ion with a positive charge because the one extra proton in the nucleus will become unbalanced by an electron in the orbit. This ejected electron get attached to the another electron of the substance forming pairs of positive and negative ions. The neutrons being neutral/ or being without any charge do not cause expulsion of orbital electrons in the matter but they can cause ionization by striking the nucleus of an atom in the substance resulting the excitation and emission of the charged particles. The energy absorbed in the form of waves causes the state of instability and thus the absorbed extra energy is decapitated by throwing off one of the orbital electrons. The additional ionization is produced by electron since it is a charged particle moving through the matter. Such radiations causing ionization are also known as ionization radiations.

Ultra violet rays- These are non- ionizing EMR with wave length between 1000 to 4000 A^0 i.e. very long wave lengths and therefore, do not penetrate the tissue appreciably but simply cause the excitation of planetary electrons which results in increased chemical reactivity inside the tissue. Thus these biological effects are only due to excitation and photochemical reactions. Because of very less penetrating power ultraviolet rays are not efficient in producing chromosomal and gene mutation as compared to ionizing radiation. These radiations are used in Palynology (pollen grains studies). So far no mutants of economic importance have been produced in higher plants with ultraviolet radiation. UV rays in mercury vapour lamps and tubes specially designed for UV treatment is carried out by exposing the desired plant material to them. UV rays treatment is relatively inexpensive.

B. Chemical Mutagens- Mustard gas and its related compounds are the most powerful chemical mutagens. They are applied to desired plant material in form of solution and induce both chromosomal and gene mutation. Some of the important chemical mutagens are as follows:

A. Mustard compounds & lachrymatory substances:
1. Mustard compounds, Sulphur mustard and Nitrogen mustard are highly mutagenic.
2. Mustard oil, Chloro-acetone, Dichloro-acetone are weakly mutagenic.

B. Alkalating agents:
1. Ethylene Oxide, Busulphan (1,4 Dimethane Sulphonoxy Butane), 1:2,3:4 Diepoxybutane, Trizine , etc.
2. Purines and Purine derivatives - Caffeine, Theophyline, Paraxanthine, Theobromine, Tetra-Methyluric acid and Adenine.

C. Others: Potassium thiocyanate, Ethyle carbamate, Formalin, Maleic hydrazide, Manganese chloride

Procedure of Mutation Breeding:

Plant material for irradiation- Any form of plant material i.e. seeds, seedlings or cuttings can be treated in any form with radiation of different kinds.

Seeds- Mature aged seeds show greater frequency of induced mutation than the fresh and dried ones. For irradiation, mature seeds with normal water content are best.

Seedlings- Usually neither too old nor too young seedlings are suitable for irradiation. The mature plants are less sensitive for irradiation.

Cuttings- In case of fruit trees, where vegetative propagation is followed, the desirable cuttings can be exposed to irradiation treatment.

Treatment- For different treatments plant material is selected and treated with particular radiation or chemicals. For x- ray irradiation the material is taken inside x- ray rooms to be exposed to x- rays. Seeds are kept in petri dishes while seedlings with pots are put before x- ray machine. For Gamma irradiation the pot grown plant are taken into the reserved space, very near the source. While for seed treatment a tray fixed in the aluminium tray tube through which the radioactive cobalt source travel when lifted from the container and in this tray the plant material is kept and subjected to desired dosage of Gamma rays.

However, for neutron treatment the seeds as well as the seedlings are exposed to neutron flux from the generator. Beta rays treatment is done with the isotopes P^{32} and S^{35} available in the form of the phosphoric acid and sulphuric acid, respectively. The seeds and seedlings to be treated for irradiation are immersed in respective solutions of desired strength for specific duration. However, in some cases the solution of radio isotopes is also applied to the soil directly.

Dosage- To treat the plant with desirable dosage is difficult for mutation breeders. The proper dose will be one which gives an appreciable frequency of beneficial mutants on selection and makes the heritability value of yield other desirable characters optimum. This dose varies from crop to crop depending upon the nature and genetical constitution of the plant material.

First irradiation generation- The irradiated material is removed immediately and sown into the field surrounded by the control material of mother strains because the storage of treated material increases the injuries in R_1 generation. The R_1 generation is grown along with the plantation of control strains all around the treated material:

i) To isolate the treated population completely from the varieties so that no interspacing may occur and hamper the observations on mutagenic effects and,
ii) To find out the effects of irradiation on irradiated plants with control ones.

Effect of treatment in R_1- The treated plants show different types of mutagenic effects. Here the higher dosage is more prominent to produce visible disturbances than the lower dosage. These effects are seen in four categories- viz. i) death ii) growth inhibition iii) morphological and developmental abnormalities and, iv) changes in genetic constitution. There is no need of further studies in the first case, while in the case of inhibition of growth and morphological and developmental abnormalities, the effects are generally temporary and the plant will recover soon.

The true changes or mutations though rare and are more useful. Thus we are more interested in the mutation rather than in other abnormal plants. The mutation effects may be due to chromosomal, cytological and gene changes.

In chromosomal mutations or polyploidy normally all the plant features are affected while in other types of mutation only few or even single characters are affected. Mutation may be either dominant or recessive but the recessive are more common. This type of mutation could not express itself phenotypically until and unless two recessive genes come close together and become homozygous. So the recessive mutation express phenotypically after a few generations.

Selection of R_1 generation: For the selection of R_1 generation all the suspected plants and plant tillers ears are picked in R_1 and for future investigation kept separate.

R_2 generation: To develop R_2 generation seeds of individual ears of R_1 are grown in progeny rows because the R_1 ears are affected independently by mutations as stated earlier under effects. However, one or three grains from R_1 plants are picked up in rows beside ear selection and sown in R_2 rows, and they are known as one plant- one grain or one plant- three grains method respectively. These methods are considered more efficient then ear -to progeny rows method because of reduction in land and labor expenses, and it is easier to distinguish the desirable mutant from the original variety in R_2.

Precautions: To avoid and eliminate every error it is good to sow 14 to 15 seeds in each progeny row which may creep in due to too many or too less seeds per row. After every tenth progeny row, a row of original variety is sown as a check for comparison. In the sowing of progenies of individual plants in separate rows by applying any method whether ear -to row or grain -to- row, facilitates the detection of mutants in R_2 in their breeding behavior.

Screening and selection in R_2 generation: In R_2 generation screening of irradiated material is started even for the dominant mutants because the recessive mutants are more frequent than dominant. As both recessive and dominant loci are rarely mutated therefore, to isolate them segregation must be permitted for letting them to come in homozygous condition so that they can be isolated. Sometimes it may be possible that due to natural intercrossing or due to loss of genetic material during sexual reproduction dominant character may appear rather than induction of true mutation. These above mentioned difficulties of recessiveness and infrequency of dominant mutants are automatically lessened in R_2 and screening becomes very easy in irradiated material.

To avoid the difficulty of low frequency of dominant mutants, a large population is taken in R_2 where efficient screening techniques have been developed in a material for instance; screening is very easy in disease resistance. Now due to increasing background and advance theoretical knowledge of mutation it is easy to control the mutation in any direct direction and induce beneficial mutations considerably.

Higher dosage of mutagen treatment seems to bring drastic changes in the morphology of the plants e.g., thickening, thinning or shortening of already existing organs. It has been observed that more efficient techniques of screening yields better chances of obtaining varied and desired kind of mutations.

The seeds from different ears of desired and best plants are collected separately**.** Sometimes some aberrant type seeds can also be mixed together for better results.

R_3 and R_4 generations: To raise R_4 generation plants to progeny row are raised with original variety in R_3 generation. The number of plants per row is 14 to 15 but it is better to have larger number of plants. If all the plants in R_3 are true to type as in R_2 they are called mutants. From them plants with high yield and slightly differing homozygous lines are selected. The true breeding lines are carried in F_4 and combined in progeny rows with original strains in control. All the inferior lines are completely eliminated in R_4. After first inspection if the homozygous appear productive in R_4 trials, the best ones are selected for full yield comparisons in R_6 and, others are kept in material collection.

Yield trials: The yield trials are started in R_5 generation at a moderate rate and only the highly useful mutants are retained. The trials may be tried in R_5 to R_7 generations regarding yield till the superior strains for desired characters for which mutation breeding was started initially, are obtained. All the techniques of crossing and other steps are same as those of conventional hybridization program.

Time taken: For obtaining desired or improved varieties the time varies depending upon the nature of improvement needed and the stage at which it is obtained.

Precautions

1. The first and foremost precaution in mutation breeding is to ensure that the isolated mutants are the original or real and, not due to intermixing, crossing or segregation.
2) The plant breeders must be well trained in all breeding techniques including mutations and very keen and sharp observer and able to pick up even the slightest variation in the treated population and include all such variations for further investigations.

Applications of mutation breeding: Mutation breeding can be used in all three types of crops like conventional breeding i.e. self-pollinated, cross- pollinated and vegetatively propagated crops.

Mutation breeding in self- pollinated crops: These crops are genetically homozygous and have very less variations. In self pollinated crops mutants can be easily detected due to purity and true breeding nature of their progeny. So on account of these characters in SPCs the mutation breeding has been most widely and common practice.

Mutation breeding in cross pollinated crops: .These crops are generally heterozygous and thus due to inter crossing within the variety and, their normal variability give rise to different types of progeny which hampers the detection of mutants in the population. It is believed that in these crops natural genetic variability is in abundance so it is totally unnecessary to use mutation breeding as a source of increasing the variation in the cross pollinated crops.

Mutation breeding in vegetatively propagated crops: The vegetatively propagated plants are highly heterozygous, hence conventional breeding procedures hardly permit the powerful improvement in these crops. In these plants sexual reproduction destroys all the valuable characters of vegetatively propagated plants. So to add new desirable characters in such crops it is always advantageous to use mutation breeding for new desirable characters.

However, application of mutation breeding is avoidable under the following problems of crop improvement:
1) When whole of the naturally occurring variability is exhausted
2) When a gene for desirable improvement is known to exist but unattainable.
3) When a desirable occurring variability is exhausted but not desirable to attain it.

Limitations- Mutation breeding is not a good device for crops improvement until one have full and proper information about the genotype of material, proper mutagens and its frequency, proper and sufficient techniques and other related factors and phenomena. For proper mutation breeding few institutions have irradiation facilities and trained personnel are available. Government too, can afford to grant such facilities only to a few institutions.

Advantages- The advantages of mutation breeding are that it produces inexhaustible variations. These created variations are not merely due to recombinations as in hybridization but they are original and newly created. This makes the plant breeders free from complete dependence on nature for raw materials.

When no improvement is possible by conventional methods whether variation is present or not, only mutation breeding can be helpful. The most significant point is that under special circumstances mutation breeding has maximum utility in crop improvement. Mutation breeding needed least time, labor, land and expenses comparatively to conventional breeding procedure.

Achievements- In India mutation breeding work had been initiated in 1935 by Ramiah *et. al*. After that individual workers made dissipated attempts in ill-equipped laboratories to produce improved strains in different crops. Two Gamma Gardens were established at Bose Research Institute, Calcutta (1959) and I.A.R.I., New Delhi (1960) that opened a new research area in crop improvement. Now the mutation facilities are available at the Bose Research Institute, Calcutta, Bhabha Atomic Research Station, Trombay, the Institute of Fundamental Research, Bombay and the IARI New Delhi, etc. In these research stations plant material can be treated and breeding

carried out at different places in Universities, Departments, Research Institutions and Farms by different workers.

In India different workers have made the efforts in different crops viz., wheat, rice, oat, barley, cotton, mustard, groundnut, sesames, jute, tomato sugarcane, etc. In wheat the main achievements are rust resistance, higher yield, induction of awns, non- lodging, ear branching and short and stiff straw, etc. In sugarcane increased yield and disease resistance are main objectives and in jute tallness of plants and early flowering have been considered. Workers and researchers are still working in many more mutant varieties in different crops which are under trial and evaluation and will be released in near future.

Conclusion: Development of improved varieties of crop plants using radiation for mutation breeding is considered as the greatest way of peaceful utilization of atomic energy for human welfare. Mutation breeding is commonly used to produce traits in crops such as larger seeds, new color or sweeter fruits that either cannot be found in nature or have been lost during evolution. It has been applied since 1930s to accelerate the process of developing and selecting new valuable agronomic traits. It is simply a new device in the breeder's tool kit.

Mutation breeding has many comparative advantages being cost effective, quick proven and robust. It is transferable, ubiquitously applicable, non- hazardous and environmentally friendly. It is commonly used in self- pollinated crops. However, the mandate of the IAEA is to seek and accelerate the contribution of atomic energy to peace, better health and prosperity of human beings throughout the world and, to foster the exchange of scientific and technical information on peaceful uses of atomic energy.

3.4 POLYPLOIDY

Generally the gamete cell contains a single genome called haploid. In the life cycle of an individual mostly the haploid gametes of both sexes i.e. male and female, after fertilization united together and forms a diploid zygote, having two genomes. After embryological development the individual attains maturity and produces haploid gametes. Thus an alternation of generation continues between haploid and diploid phases. Some organisms have more than two genomes (2x) and is called a polyploid (Greek word- poly means much/ many, ploidy means form or number). In nature there are a number of plant genera constitute a euploid (even number of genome) series of chromosome number *i.e.*, Rosa (Rose) include species with the somatic numbers as 14, 21, 28, 35, 42 and 56. These all are the multiple of 7. Hence this shows the euploid series of monoploid number 7 and gives diploid (14), triploid (21), tetraploid (28), pentaploid (35) haxaploid (42) and octaploid (56). These belong to polyploidy category except the diploid which is the normal one. Most of the important crops, ornamental plants and flowers are the best examples of polyploids *e.g.* wheat (hexaploid 6x), strawberry (octaploid 8x), etc.

3.4.1 Causes of polyploidy: The major cause of polyploidy is found to be the non-disjunction of sister chromatids during the meiotic recombination events. Prior to meiosis chromosome number doubles followed by chromosome separation during gamete formation. Autopolyploids results from failure of segregation of chromosomes during gamete formation. Somatic doubling in apical meristem is another cause and results from mitotic divisions.

Allopolyploids are formed when hybridization occurs between different genome sets. The initial hybrid diploid products with different sets of chromosomes are usually sterile and genetically unstable. However, this can undergo meiosis prior to which it doubles the chromosome number and there are chances to create a genetically stable polyploidy.

Comparatively to natural polyploids artificially generated polyploids have been found to be useful both in research as well as economic purposes. Colchicine, the chemical which interfere with meiosis spindle formation is widely used in development of new crop varieties.

3.4.2 Types of polyploidy: There are three different types of polyploidy:

i) Autopolyploidy
ii) Allopolyploidy
iii) Autoallopolyploidy

i. Autopolyploidy: The polyploids which consist of same basic set of chromosomes in its multiple are known as Autopolyploids i.e. if a diploid species consists two similar sets of chromosomes of genomes (AA), then its autotriploid have three similar genome (AAA) and an autotetraploid will have four similar genome (AAAA), or simply when the presence of multiple sets of chromosomes derived from a single genotype. This type results occurs mostly from somatic doubling either in zygotes or in the cells of apical meristem region. Since this involves doubling of the existing number of chromosomes, the resultant ploidy is always even. The chromosomes are similar and hence can lead to multivalent pairing at meiosis. The allelic relationship is difficult to be deciphered making genetic analysis little complex.

Origin and production of autopolyploids: The autopolyploids may originate naturally or may be produced artificially also. *Cynodon dactylon* or doob grass is the common example of natural autopolyploids, mostly cultivated in U.P. and Bihar. Gupta established the autotriploid status of *Cynodon* from its meiotic behavior. Seedless varieties of water melons, sugar beet, tomato, banana, grapes, etc. are some common examples of autopolyploid crop plants mainly produced by artificial methods. Similarly many important crop plants i.e. corn (*Zea mays*), rye (*Secale* cereal), berseem (*Trifolium alexandrium*), red clover (*Trifolium pretense),* marigold (*Tagetes*), Snapdragons (*Antirrhinum),* grapes, apple and evening primroses (*Oenothera lamarckiana),* etc.

Induced/ artificial autopolyploidy: The autopolyploid have been induced in many plants and animal cells artificially by means of chemicals e.g. colchicines, chloral hydrate, sulphanil amide,

mercury chloride, etc. and, by temperature checks, radioactive substances for instance X- rays and radium.

ii. Allopolyploidy: Allopolyploids are typically derived from hybridization between two or more distantly related species and combine divergent genomes with their own chromosome complements. Allopolyploids include important crops such as wheat (*Triticum aestivum*), cotton (*Gossypium*) and canola (*Brassica napus*) and all have improved agricultural traits relative to their diploid progenitors. Wheat was domesticated about 10, 000 years ago, and bread wheat (*Triticum eastivum)* has a genome composition of AABBDD, which arose by interspecific hybridization between *T. turgitum* (AABB) and *Aegilops tauschii* (DD).

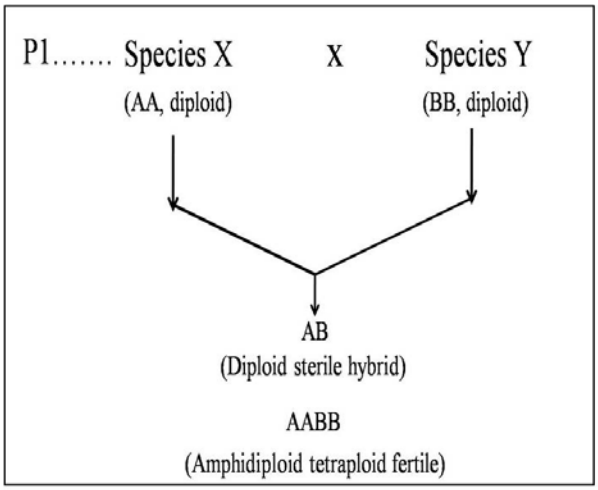

Fig. 3.10: Formation of an amphidiploid tetraploid

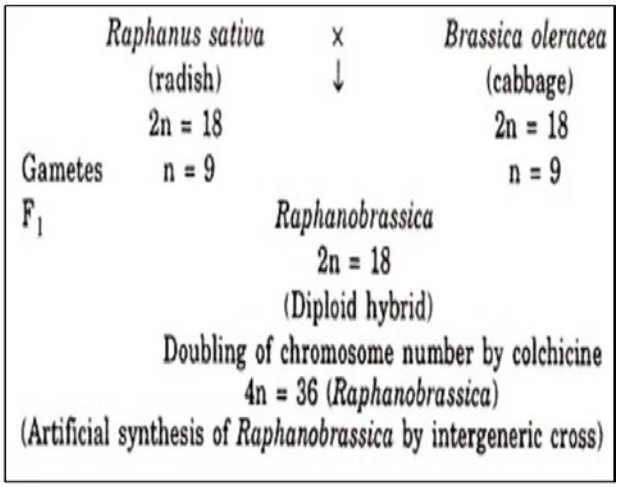

Fig. 3.11: Artificial synthesis of Raphanobrassica

Allopolyploidy results due to the doubling of chromosome number in a F_1 hybrid derived from two distinct species. This is also known as amphiallopolyploid tetraploid as for example (**Fig. 3.10**) there are two species X and Y. Suppose A species represent a set of chromosome or genome in species X and B represent another set of chromosome or genome in Y. In F_1 hybrid there is doubling of chromosomes with two A and B genomes. The most important example of Allopolyploidy is *Raphanobrassica* (**Fig. 3.11**). A cross between radish (*Raphanus sativum* 2n = 18) and cabbage (*Brassica oleracea* 2n = 18) when crossed yielded a sterile (diploid) hybrid (Karpechenko, 1927). He found certain fertile plants among these sterile F_1 hybrids which contain 36 chromosomes. These fertile plants are known as *Raphanobrassica*.

Synthesized allopolyploids: It is revealed by some cytogeneticists that how the allopolyploids originated naturally. At present many important and significant crop varieties have been synthesized by scientists in recent past. Artificially synthesized allopolyploids are excellent material to understand early events following hybridization because the exact progenitors are known. Methods for synthesizing allopolyploids have been developed and artificially synthesized allopolyploids have been widely generated.

i) *Triticum spelta*: McFadden and Sears (1944) and, Kihara (1944) synthesized the hexaploid wheat *Triticum spelta* artificially. For this they crossed an emmer wheat (hulled wheat), a type of awned wheat which is tetraploid, *Triticum dicoccoides* (tetraploid: 2n = 28) with goat grass, *Aegilops squarrosa* (diploid; 2n= 14). In F_1 hybrids were sterile and doubling the chromosome number resulted artificially synthesized hexaploid wheat. These were again crossed with naturally occurring *T. spelta*. The F_1 hybrids were completely fertile. The result proved that in past the hexaploid wheat must have originated due to natural hybridization between goat grass and tetraploid wheat by subsequent chromosome doubling method.

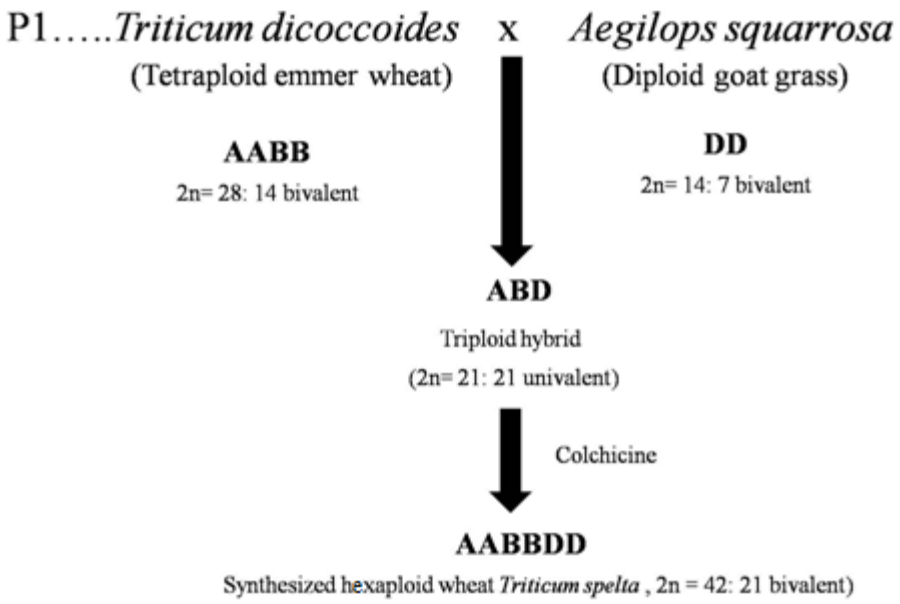

Fig. 3.12: Artificial synthesis of hexaploid wheat

ii) *Gossypium hirsutum:* Another interesting example of allopolyploidy is the New World Cotton plant (**Fig. 3.13**). The Old World Cotton, *Gossypium herbaceum* has 13 pairs of chromosomes and upland cotton or American cotton also contains 13 pairs of chromosomes. American cotton and old world cotton was crossed by Beasley and, doubled the chromosome number in the F_1 hybrid thus producing allopolyploids which resembled the cultivated new cotton. This new cotton crossed with it and gave fertile F_1 hybrid. Thus these results suggested that the new world cotton, *Gossypium hirsutum* would have originated from two species *i.e. G. herbaceum* (2n = 26) and *G. raimondii* (2n = 26).

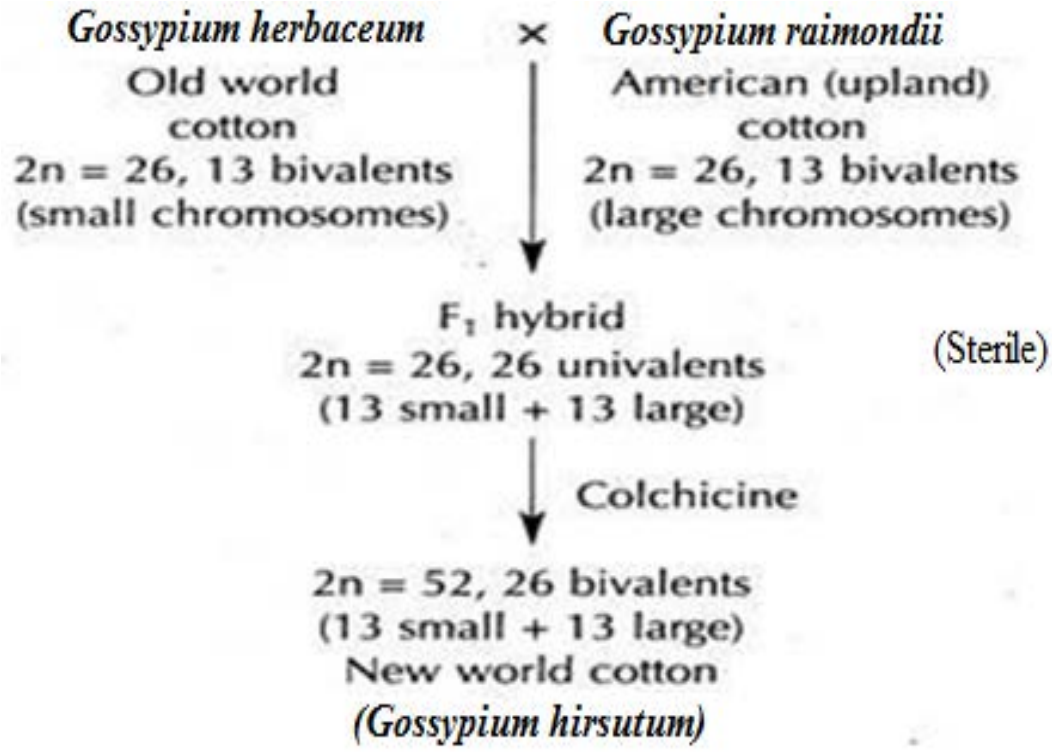

Fig. 3.13: Example of allopolyploidy; the New World Cotton plant

iii) Triticale: *Triticosecale wittmack* has been developed in recent years and known as first human made cereal. It is cultivated throughout the world and occupies about one million hectare of global land for commercial use. It is an artificial allopolyploid which has been derived by crossing *Triticum* (wheat) and *Secale* (rye). *Triticum* is a tetraploid (2n = 4x = 14) or may be hexaploid (2n = 6x = 42) or octaploid (2n = 8x = 56) respectively as follows (**Fig. 3.14** and **Fig. 3.15**):

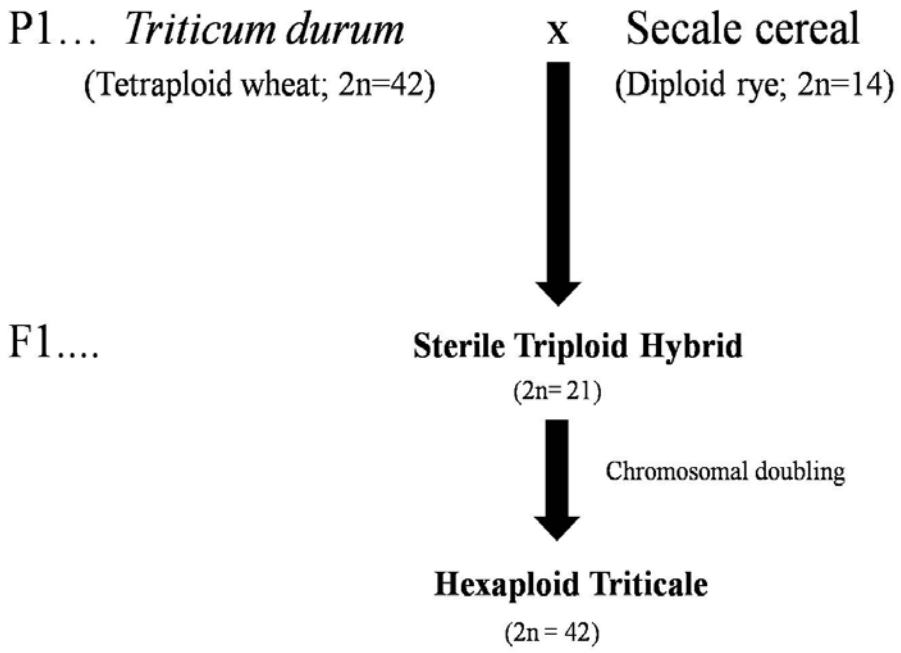

Fig. 3.14: Artificial synthesis of a hexaploid triticale

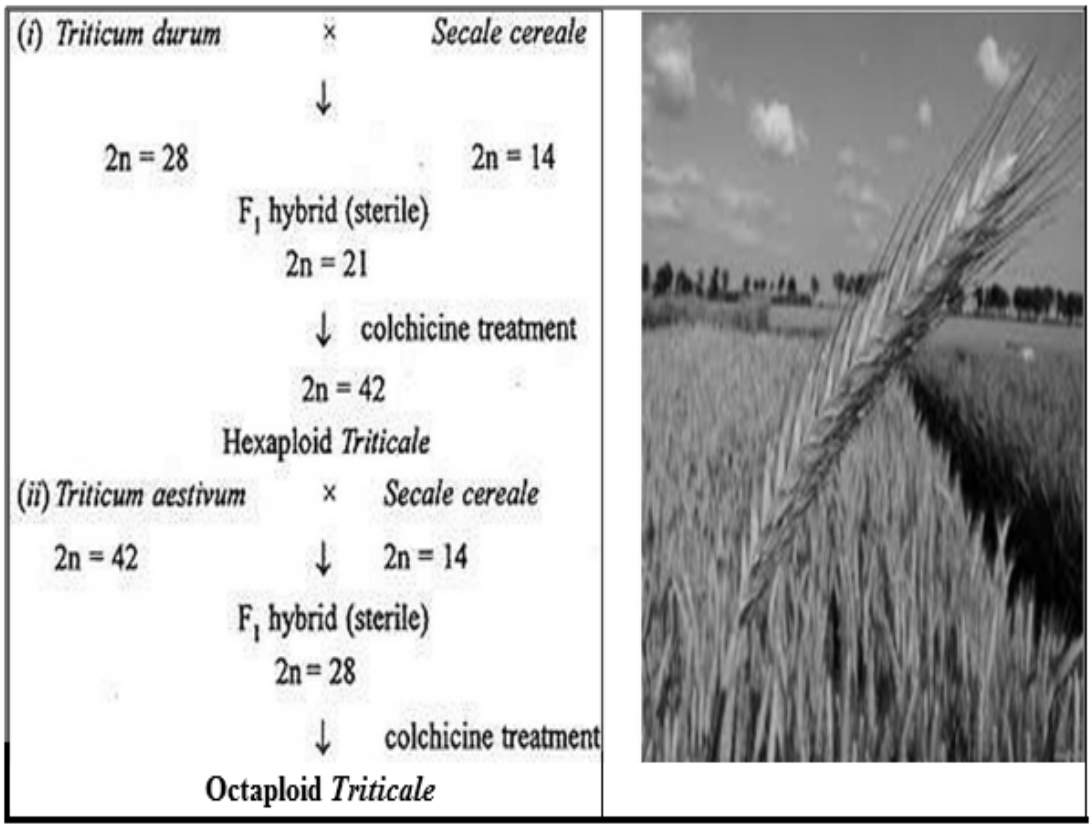

Fig. 3.15: Artificial synthesis of octaploid Triticale

3.4.3 Evolutionary Significance of Polyploidy: Polyploidy is a successful evolutionary strategy in a plant as follow:

1. Polyploidy is more common in plants rather than in animals because plants can generally avoid the meiotic problems of polyploidy easier than most animals.

2. Some plants can reproduce vegetatively and hence allowing more time for somatic doubling event to occur or provide longer time for somatic doubling of chromosomes that will produce amphipolyploidy.

3. In many plants the process of fertilization is fulfilled by wind or insects pollinators and thus they have more opportunity for hybridization.

4. Recently polyploidy has been used in agriculture to produce economically important crops such as ***"seedless"*** as well *as* ***"Jumbo"*** varieties of crops *i.e.* seedless watermelon is a triploid, its seeds are generally sterile and do not develop. It is produced by growing seeds from the cross between a diploid variety and a tetraploid variety. While *jumbo* ***McIntosh apples*** are tetraploid (Tamarin, 2002).

3.4.4 Conclusion: The various breeding methods as mentioned above have been developed on the basis of nature of crop propagation i.e. either it may reproduce sexually through self or cross- pollination or through vegetative propagation. The main basis of application of crop method to be used in a crop at any place depends on the amount of variation present in that particular crop in the past. The first method to improve the crop is the selection, if no breeding work has been done so far in a crop. It may be mass, pure line or clonal, pedigree, bulk or back cross selection depending upon the type of propagation. If the variation is not enough for selection then the next method of crop improvement is hybridization and selection from hybridization can meet the desired needs. However, when recombination of hybridization may fail to obtain variation and improvement in a crop, then the desired characters can be achieved by plant introduction and acclimatization. The desired material is introduced from outside and either utilized as such or incorporated in the local material. If all these methods are not successful and effective to obtain any desired variation, the last tool in hand is mutation breeding. Here the variation is created artificially by applying physical or chemical mutagens and used to produce new superior strain for future.

3.5 SUMMARY

Plants both domesticated as well as introduced, show considerable degree of variations with respect to different characters. Some of these plants are superior whereas the others are inferior in performance. The process of selection of superior plants is an important method for the improvement of cultivated plants, which lead to the development of new varieties with more advantageous and superior characters. After selections *i.e.* mass selection, pure line selection and

clonal selection, the most frequently employed plant breeding technique is hybridization. The aim of hybridization is to bring together desired traits found in different plant lines into one line via cross pollination. Heterosis is achieved by crossing distant inbred lines of crop plants, whereas mutation refers to sudden heritable changes in the phenotype of an individual. Mutations do occur in nature either as spontaneous mutation or can be artificially introduced by various mutagenic agents known as induced mutation. Mutation breeding is the simple, quick and best way when a new character is to be induced in vegetatively propagated crops. Applied polyploidy technique is scientifically quite sound and plays significant role to raise economically important varieties of cereals, vegetables, fruits and animals.

3.6 GLOSSARY

Acclimatization: The process by which a population adjusts and modifies itself to survive and reproduce normally under changed environmental or stress conditions.

Backcross: The cross of an F_1 hybrid with either of the parents.

Breeding: The art and science of changing and improving heredity of living organisms.

Bud Selection: The form of clonal selection in which the bud is made the unit of selection.

Clone: A group of plants produced through vegetative propagation from a single plant. It represents exact multiple copies of a genotype.

Colchicine: An alkaloid extracted from seed of *Colchicum autumnale* that destroys spindle apparatus during mitosis and thus doubles chromosome number.

Emasculation: Manual removal of the anthers from a flower to make it ineffective for producing pollen.

Hybrid: The progeny of a cross between two or more individuals, plants or animals of unlike genetical condition.

Hybridization: A method of crop improvement in which two or more plants of unlike genetical constitution differing in one or more characters are crossed together.

Induced Mutation: The mutation artificially produced with the help of mutagens.

Mass Selection: The method of selecting plants on the basis of their phenotypic performance to bulk the seed in the form of new population.

Mutagen: An agent that can induce mutation.

Mutation: A sudden heritable change in a chromosome. It may involve change either in single gene or part of chromosome.

Pedigree Selection: A record of ancestry of an individual, family or strain.

Phenotype: External appearance of an organism as contrasted with its genetic makeup or genotype for particular character.

Pure line: The progeny of a single homozygous individual produced through self- fertilization.

Male Sterility: The conditions in which the pollen is not produced or is non- functional for pollination.

Multiple Cross: A cross among many inbreds with pollination between desired lines.

Three Way Cross: A cross between a single cross used as female and an inbred used as male *i.e.* (A x B) x (C x D).

Single Cross: A cross between two inbred A x B.

Variety: A group of similar plant within a species which are distinctly different for some structural features and performance from other varieties of the same species.

3.7 SELF-ASSESSMENT QUESTIONS

3.7.1 Fill in the blanks:

1. Mass selection is the simplest---------- and ----------method of crop improvement?
2. Mass selection is used in ----------and ----------plants?
3. Most frequently employed plant breeding technique is ----------?
4. In pure line selection the expected results is a set of genetically---------- lines?
5. A cross {(A x B) X (C x D)} is known as----------cross?
6. [(A x B) x C] represents----------cross?

Answer Key: 1. common, oldest, 2. Self and cross-pollinated, 3. Hybridization, 4. (Homozygous), 5. Double cross, 6. Three way cross.

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3.10 TERMINAL QUESTIONS

3.10.1 Long answer type questions:

1. What are the different methods of crop improvement and when and where are they used in particular crop?
2. Define artificial selection and what its different types?
3. What are the different hybridization methods for self and cross - pollinated crops?
4. What do you mean by mutation breeding and what is its main purpose?
5. What do you mean by polyploidy breeding, what are its different kinds on the basis of origin, define briefly?
6. Define different suitable methods of plant breeding applied for self- pollinated and cross-pollinated crops?
7. Mutation breeding and polyploidy have made evolutionary changes in modern era. Comment.
8. Describe the differences in bulk method and back cross method.
9. Define the following terms:
   a. Pure- line selection
   b. Clone
   c. Pedigree method
   d. Bulk method
   e. Back cross method
   f. Mutation Breeding
   g. Allopolyploidy
   h. Autopolyploidy
10. What are the differences between mass selection and pure line selection?
11. Write about different steps applied in hybridization technique?
12. What type selection methods are used for the improvement in vegetatively propagated crops?
13. Define the merits and demerits of mass selection?
14. Define the achievements of mass selection?

BLOCK-2- PLANT BREEDING FOR SPECIFIC

UNIT-4 MALE STERILE AND HETEROSIS BREEDING FEATURES

4.1 OBJECTIVES

After reading this unit students will be able-

- To study male sterility
- To learn about heterosis breeding features
- To study applications of male sterility and heterosis breeding in crop improvements.

4.2- INTRODUCTION

Male sterility is a **phenomenon** where the **male** reproductive parts of the **plants** do not develop normally and fail to participate in sexual reproduction. The **male sterility** is of different kinds and can arise through a number of biological abnormalities.

Male sterility is an important trait and plays a key role for hybrid **crop** production in agricultural industry field. Functional **male sterility** can be utilized in the production of hybrid seed. During **plant breeding** for hybrid **crop** production, a lot of genetic diversities are created.

4.3- MALE STERILE

Discovery of Male sterility

Koelreuter (1763) first reported male sterility phenomenon in flowering plants. Later on numerous cases of male sterility in angiosperms were reported. Allard (1960) and Duvick (1966) presented a good account of male sterility in flowering plants. More recently, work on male sterility has been reviewed by Frankel and Galun (1977) and Kaul (1988). Male sterility refers to a condition in which pollen is either absent or non-functional in flowering. The main features of male sterility are briefly presented below:

1. Male sterility is an important outbreeding device which prevents autogamy and permits allogamy. In other words, male sterile plants produce seeds only on cross pollination with functional pollens of other plants.
2. Presence of male sterility leads to heterozygosity in a species as it promotes out breeding and reduces homozygosity due to elimination of inbreeding.
3. Male sterility results from the action of nuclear genes or cytoplasmic genes or both. It is caused due to pollen or anther abortion.
4. Male sterility occurs in nature through spontaneous mutations as well as can be induced artificially by chemical or physical mutagens. Ethidium bromide has been found effective in inducing cytoplasmic male sterility in some plants.
5. Male sterility can be observed in all diploid species of crop plants both wild and cultivated if properly investigated. Male sterility has been reported in several crop plants. Male sterility has some similarities and some dis-similarities with self incompatibility.

Types of male sterility:-

The male sterility is of five types: (1) genetic male sterility (2) cytoplasmic male sterility, (3) cytoplasmic genetic male sterility, (4) chemical induced male sterility and, (5) transgenic male sterility. Almost all crop plants possess male sterility if investigated properly. A brief description of five types of male sterility is given below:

1. Genetic Male Sterility (GMS)

The male sterility caused by nuclear genes is termed as genic or genetic male sterility. This type of sterility has been reported in several crop plants like barley, cotton, cucurbits, lucerne, maize, sorghum, sugar beet, tomato and wheat,. The main features of genetic male sterility are:

TABLE1: Comparison of self incompatibility and male sterility

Self incompatibility	**Male sterility**
Similarities	
1. It is an important out breeding mechanism.	1. It is also an important out breeding mechanism.
2. It prevents auto gamy and promotes allogamy.	2. Same as incompatibility.
3. It is found in nature.	3. Also found in nature.
4. It is used for hybrid seed production.	4. Also used for development of hybrids.
Dissimilarities	
1. Pollen is functional.	1. Pollen is absent or non-functional.
2. It result, due to morphological, genetic, physiological and biochemical causes.	2. It results due to genetic, cytoplasmic or both causes.
3. It may be heteromorphic, homomorphic, gametophytic, or sporophytic.	3. It may be genetic, cytoplamic or cytoplasmic genetic.
4. Artificial induction is difficult.	4. Artificial induction is easy.

1. Male sterility genes are usually recessive (Lewis and Crow 1956) and rarely dominant.
2. In majority of cases, sterility is caused by single gene. However in few cases two or more genes control male sterility.
3. This system consists of two types of lines viz. A line and B line. A line refers to genetic male sterile line (mm) which is used as female parent in hybrid seed production. B line is heterozygous fertile (Mm) line which is similar to A line except for sterility. B line is used to maintain sterile line (A). Thus A and B lines are isogenic lines with a difference of fertility/ sterility locus.
4. The genetic male sterile line is maintained by crossing recessive male sterile plants (mm) with heterozygous male fertile plans (Mm). Such cross will yield 50% sterile plants and 50% fertile plants. The male sterile plants are used as female parents in the development of hybrids. The fertile plants are rouged out. Crosses between recessive male sterile plants and heterozygous male fertile plants are made every year for maintaining the male sterility.

Merits and Demerits:

Genetic male sterility is usually recessive and monogenic. Hence, fertility restoration in the hybrid and the crossing plans are relatively easy. GMS can be used for the production of hybrid seeds in both seed propagated crops and vegetatively propagated species. Genetic male sterility generally does not have undesirable agronomic characters. Moreover, it requires less area and labour because the breeder has to maintain only A and B lines.

There are two main disadvantages of genic male sterility. Firstly, it is less stable. GMS can sometimes become fertile at low temperature. For example, in rice the sterile plants become fertile at temperature below 23^0C. The sterility which in influenced by the temperature is called thermo-sensitive male sterility. Sometimes the male sterility is influenced by day length. In rice, long day condition leads to male sterility and short day produces male fertility provided the temperature ranges between 23- 29^0C. Such male sterility is called photosensitive male sterility. In cotton, below 12^0C sterile plants sometimes become fertile. The second demerit is that 50% plants are fertile which have to be removed every year. This increases the cost of hybrid seed.

TABLE 2: List of some crop plants in which male sterility is found:

Plant family	Name of the plant		Type of male Sterility found
	English	Scientific	

Poaceae	Barley	*Hordeum vulgare*	GMS
	Wheat	*Triticum aestivum*	GMS.CGMS
	Maize	*Zea mays*	GMS.CGMS
	Sorghum	*Sorghum bicolour*	GMS.CGMS
	Pearl millet	*Pennisetum americanum*	CGMS
	Rice	*Oryza sativa*	GMS.CGMS
	Sugarcane	*Saccharum officinarum*	CGMS
Fabaceae	Lucerne	*Medicago sativa*	GMS
Asteraceae	Sunflower	*Helianthus annus*	GMS. CGMS
Cucurbitaceae	Watermelon	*Citrullus vulgaris*	GMS
	Muskmelon	*Cucurbita moschata*	GMS
	Pumpkin	*Cucurbita maxima*	GMS
Malvaceae	Cotton	*Gossypium hirsutum*	GMS,. CGMS
Solanaceae	Tomato	*Lycopersicon esculentum*	GMS, CGMS
	Tobacco	*Nicotiana tabacum*	GMS, CGMS
Liliaceae	Onion	*Allium cepa*	GMS, CGMS
Chenopodiaceae	Sugarbeet	*Beta vulgaris*	GMS, CGMS
Linaceae	Linseed	*Linum usitatissimum*	GMS
Apiaceae	Carrot	*Daucus carota*	GMS

GMS= genetic male sterility; CMS = cytoplasmic male sterility

CGMS= Cytoplasmic genetic male sterility

2. Cytoplasmic Male Sterility (CMS)

The pollen sterility which is controlled by cytoplasmic genes or plasmagenes, is known as cytoplasmic male sterility. It has been reported in different crop plants. The main features of cytoplasmic male sterility are given below.

1. Plants carrying particular type of cytoplasm are male sterile but will produce seeds if pollinators are present. The F_1 seeds (cross seeds) produce only male sterile plants, because their cytoplasm is derived entirely from the female gamete.
2. This system consisting of A and B lines is male sterile and B is male fertile. For all other characters A and B lines are similar or isogenic.
3. The cytoplasmic male sterile line is maintained by crossing of A line with B line.
4. Cytoplasmic male sterility cannot be utilized for hybrid seed production without the use of restorer line because F1 seeds produce only male sterile F1 plants. The restorer line only can provide fertility in F1 hybrids. Cytoplasmic male sterility can be used in crops where grain is not the economic product.
5. Cytoplasmic male sterility is not influenced by environmental factors such as low or high temperature. In other words, the sterility is stable.

Merits and demerits:-

The main advantage of CMS is that it is highly stable and is not influenced by environmental conditions such as temperature and day length. A cross between A and B line will always produce sterile F1. Moreover, CMD requires less area because the breeder has to maintain A and B lines only.

CMS has limited applications. It cannot be used for development of hybrids in those crops where seed is the economic product. It can be used only in asexually propagated species such as sugarcane, potato and forage crops. CMS is solely governed by plasma genes. Therefore, it is impossible to restore fertility in the hybrids. Sometimes, CMS line has inferior agronomic performance.

3. Cytoplasmic Genetic Male Sterility (CGMS)

When pollen sterility is controlled by both cytoplasmic and nuclear genes, it is known as cytoplasmic genetic male sterility. This type of male sterility was first discovered by Jones and Davis in 1944 in onion. Now CGMS has been reported in several crops. The main features of cytoplasmic genetic male sterility sterility are given below:

1. The male sterility is controlled by the interaction of cytoplasm and nuclear genes.
2. This system includes A, B and R lines. A is male sterile line; B is similar to A in all features but is male fertile and R restores the fertility in the F1 hybrid and hence is called restorer line. Since B line is used to maintain the fertility, it is also referred to as maintainer line.
3. Cytoplasmic male sterile line can be maintained by crossing the male sterile cytoplasmic line with male fertile cytoplasmic line. In other words cross of A line with B line is used for maintaining the CGMS line as given below:

(ms/ms) S * (ms/ms) F ---- (ms/ms) S

Male sterile male fertile male sterile

Merits and demerits

Cytoplasmic genic sterility is widely used for hybrid seed production in both seed propagated and vegetatively propagated species. The CGMS is highly stable and reliable. It is not affected by environmental factors. There are two main disadvantages of CGMS. Firstly it requires more *viz*., A, B and R lines. Moreover, sometimes CGMS line has inferior agronomic performance.

A brief comparison of three types of male sterility is presented in Table 3.

TABLE 3: Comparison of three types of male sterility in crop plants

S.No	Particulars	Genetic male sterility	Cytoplasmic male sterility	Cytoplasmic genetic male sterility
1.	Control	Controlled by nuclear genes.	Controlled by cytoplasmic genes	Controlled by both nuclear and cytoplasmic genes
2.	Material included	Consists of A and B lines.	Consists of A and B lines	Consists of A, B & R lines
3.	Effect of temperature	May become fertile at low temperature.	No effect of temperature	No effect of temperature.
4.	Maintenance	Maintained by mating sterile plants with heterozygous male fertile plants.	Maintained by crossing of A line with B line.	Maintained by crossing of A line with B line.
5.	Uses	Used for production of hybrids in both seed and vegetatively propagated	Used for development of hybrids in vegetatively propagated crop	Can be used in both seed propagated and vegetatively propagated species.

	Demerits	crops.	plants.	
6.			It cannot be used in seed propagated plants because the F, is sterile	It requires handling of three types of material i.e. A,B and R lines.
	Examples	The male sterile and fertile plants are observed in 1:1 ratio. The fertile plants have to be removed in hybrid seed production plot.		
7.		Barley, cotton, lucerne, muskmelon, pumpkin, rice, sunflower, tomato, tobacco, water-melon, wheat, etc.	Onion, sugarcane, forage crop, etc.	Cotton, maize, Pearlmillet, rice, Sorghum, sugar beet, sunflower, tobacco, tomato, wheat, etc.

4. Chemically Induced Male Sterility (CIMS)

Male sterility originates in nature as a result of spontaneous mutations. In some species, male sterility has been derived from wild species through interspecific hybridization. It can also be induced through various chemicals. The chemicals which are used for induction of male sterility are referred to as male gametocides. The main difference between mutagen induced male sterility and gametocide induced male sterility is that the former is heritable and the latter is non-heritable. Gametocide induced male sterility has three main advantages.

1. It is a rapid method of developing male sterile line. The backcross method takes four to five years for transfer of male sterility from one genotype to another. By the use of gametocides, male sterility can be induced in one season.
2. This method is less laborious and less expensive than backcross method.
3. There is no need of maintaining A, B and R lines. The genotypes which have to be used as female parent in the development of hybrid, can be treated with male gametocide to suppress pollen formation resulting in induction of male sterility.

Some chemicals have been used for induction of male sterility in various crop plants. In China, chemically induced male sterile lines have been used for development of hybrids in rice and these hybrids have been released for commercial cultivation. An ideal gametocide should have following properties (Chopra, 1985; Sneep and Hendriksen,1979).

1. It should be selective in inducing male sterility without affecting ovule fertility. It should not be mutagenic.
2. It should give consistent results. In other words the results should be reproducible.
3. It should be economical and its method of application should be simple.

4. It should be safe for the use and should have minimum side effects on plant growth.

List of gametocides found effective on some crop plants:

Male gametocide	Crop species on which found effective
Sodium methyl arsenate	rice (used in China)
Zinc methyl arsenate	rice (used in China)
Napthalene acetic acid (NAA)	cucurbits
Gibberellins	lettuce, maize, onion, rice, sunflower
Maleic hydrazide	cucurbit, onion, tomato, wheat
FW 450	cotton, groundnut, sugarbeet, tomato
Ethrel	rice, sugar beet, wheat

The currently available male gametocides have following main drawbacks (Sneep and Hendriksen, 1979)

1. Pollen abortion is incomplete and erratic.
2. Treatments are effective only at a specific stage of crop growth and effect is short lived. This requires repeated treatment.
3. Ovule fertility is also adversely affected which leads to low seed setting.
4. Present male gametocides have several harmful side effects, such as deformation of leaf, stunting of plant growth and sometimes wilting.
5. High cost of gametocides and their repeated applications make their use uneconomical.

The effectiveness of male gametocides for large scale application in practical plant breeding is not yet established. This area further needs intensive research to reach concrete conclusions. Mutagens also induce male sterility. Ethidium bromide (EB) has been used for induction of cytoplasmic male sterility in pearl millet (Burton and Hanna 1976) and barley (Minocha *et al.* 1983).

5. Transgenic Male Sterility

The foreign gene (transgene) is also used for induction of male sterility. The male sterility induced by the technique of genetic engineering is called transgenic male sterility. The gene responsible for inducing male sterility is generally used from micro organisms. This type of male sterility comes under genetic male sterility and is heritable. Transgenic male sterility has been induced in tobacco and rapeseed by transferring a gene from *Bacillus amyloliquefaciens* (it is a non pathogenic gram positive, rod shaped soil bacteria). In this system two types of transgenes are used, one gene barnase causes male sterility hence this gene is integrated in A line. Another gene barstar suppresses the male sterility gene barnase and leads to restoration of fertility. This gene is transferred to restorer line. When a cross is made between A and R line the F_1 is fertile. This is an expensive method of obtaining male sterility. Moreover maintenance of the male sterility is a problem. This method is likely to be used for commercial hybrid seed production of various crops is the near future. Barnase gene is cytotoxic killing the tapetal cells

thus preventing pollen development and therefore causes genetic male sterility (GMS). Barstar is a chimeric tapetal cell specific ribonucleic inhibitor gene and used to restore male fertility.

Inheritance of male sterility:

The pattern of inheritance differs for three types of male sterility. Genetic male sterility is normally controlled by recessive genes. A cross between male sterile and male fertile strains produces male fertile F_1. The F_1 on selfing gives rise to 3 fertile and 1 sterile plants in F_2 generation. When F_1 is backcrossed with male sterile line, it produces sterile and fertile plants in 1:1 ratio. The fertile plants are heterozygous. This type of male sterility is maintained by sib mating of sterile (msms) and fertile (msms) plants. This always produces 50% fertile and 50% sterile plants. For hybrid seed production, the fertile plants are removed. The fertile plants are used only for maintaining the sterility.

In case of cytoplasmic male sterility, when a cross is made between male sterile and male fertile plants, the F_1 is always male sterile. The male sterile line is called A line and male fertile line is called B line. A and B are isogenic lines which differ only for one locus. The cytoplasmic sterility is maintained by crossing of A line with B line. Cytoplasmic male sterility can be used for hybridization in vegetatively propagated crops like sugarcane and potato where true seed is not of economic importance because the seed produced by the use of cytoplasmic male sterile line would give rise to sterile progeny.

4.4- HETEROSIS BREEDING FEATURES

The superiority of F_1 hybrid over both its parents in terms of yield or some other characters or heterosis is increased vigours, growth, yield or function of a hybrid over the parents, resulting from crossing of genetically unlike organisms. The term heterosis was first coined by Shull in 1914. Generally heterosis manifested as an increase in vigour, size, growth, yield or some other characteristics. But in some cases, hybrid may be inferior to the weaker parent this is also regarded as heterosis.

The superiority of F_1 is estimated over average of the two parents (mid parent). This practise has found some acceptance particularly in the practical studies. However, in practical plant breeding the superiority of F_1 over mid parent is of no use since it does not offer the hybrid any advantage over the better parent. Therefore, average heterosis is of little or no use to the plant breeder. More generally, heterosis is estimated over the superior parent such heterosis is referred as true heterosis.

However, the commercial usefulness of a hybrid would primarily based on its performance in comparison to the best commercial variety. In many cases the superior parent may be inferior to the best commercial variety. In such cases, it will be desirable to estimate heterosis in relation to the best commercial variety that is commonly known as economic or useful heterosis. Economic heterosis is the only estimate of heterosis which is of commercial or practical value. Powers

(1944) suggested that, the term heterosis should be used only when the hybrid is either superior or inferior to both the parents.

Methods for Estimation of Heterosis:

Heterosis is estimated in three different ways:
1) Mid parent heterosis
2) Better parent heterosis
3) Standard heterosis

1) **Mid Parent Heterosis:**
When the heterosis is estimated over the mid parent *i.e.* mean value or average of the two parents is known as mid parent heterosis. It is also known as average heterosis or relative heterosis and calculated by using formula:

$$\text{Mid Parent Heterosis} = \frac{F_1 - MP}{MP} \times 100$$

Where F_1 is mean of F_1 and MP is mean of two parent

2) Better Parent Heterosis:
When the heterosis is estimated over the better parent is known as better parent heterosis. It is also known as heterobeltiosis and calculated by using formula:

$$\text{Heterobeltosis} = \frac{F_1 - BP}{BP} \times 100$$

Where BP is the mean of better parent

The term heterobeltiosis was used by Bitzer *et al.* (1968) to describe the improvement of heterozygote over the better parent of the cross.

3) Standard Heterosis:
It refers to the superiority of F_1 over the standard commercial check variety. It is also called as economic heterosis or useful heterosis and calculated by using formula. -

$$\text{Standard Heterosis} = \frac{F_1 - \text{Check variety}}{\text{Check variety}} \times 100$$

Heterosis leads to increase in yield, reproductive ability, adaptability, disease and insect resistance, general vigour, quality, etc. For most of the characters, the desirable heterosis is positive. But for some characters like earliness, height in cereals and toxic substances are negative heterosis.

Theories of Heterosis:

There are two main theories which have been used to explain the mechanism of heterosis. One is the dominance hypothesis and the second is over dominance hypothesis. The epistasis is also considered to be associated with heterosis. Thus, there are three possible genetic causes of heterosis *viz.*

1) Dominance,
2) Over dominance
3) Epistasis

These are briefly discussed below:

1**) Dominance Hypothesis:**

This theory was proposed by Davenport (1908) Bruce (1910) and Keeble and Pellew (1910). This is the most widely accepted hypothesis of heterosis. According to this hypothesis, heterosis is the result of the superiority of dominant alleles, when recessive alleles are deleterious; here the deleterious recessive genes of one parent are hidden by the dominant genes of another parent and the hybrid exhibits heterosis. Both the parents differ for dominant genes. Suppose genetic constitution of one parent is AABBccdd and that of another as aabbCCDD. A hybrid between these two parents will have four dominant genes and exhibit superiority over both the parents which have two dominant genes each. Thus heterosis is directly proportional to the number of dominant genes contributed by each parent.

AABBccdd	X	aabbCCDD	-------)	AaBbCcDd
Parent 1		Parent 2		Hybrid

Objections:

There are two objections to dominant gene hypothesis.

i) If it is true it is should be possible to obtain pure heterotic individuals in F_2 which are homozygous for all the dominant genes. Jones (1917) provided explanation for this. He suggested that there may be linkage between some favourable dominant genes and some unfavourable recessive genes and as a result it is not possible to obtain true breeding homozygous individual for all dominant genes to F_2 generation. He proposed dominance of linked gene hypothesis to explain heterosis.

ii) If the heterosis is due to dominance, the F_2 curve should be skewed towards dominant genes, but the curve of F_2 is found always smooth and symmetrical not skewed. Collins (1921) provided explanation for the objections. He suggested that trial like yield is governed by large number of genes or poly genes which exhibit continuous variation resulting in symmetrical distribution of genes.

2) Overdominance Hypothesis:

It was independently proposed by Shull and East in 1908 and supported by Hull (1945). This theory is called by various names such as stimulation of heterozygosis, cumulative action of divergene alleles, single gene heterosis, super dominance and over dominance. Though this theory was proposed by Shull and East in 1908, the over dominance was coined by Hull in 1945 working on maize. This term is now in common use.

According to this hypothesis heterosis is the result of superiority of heterozygote over its both homozygous parents. Thus heterosis is directly proportional to the heterozygosis. The superiority of heterozygote over both homozygotes may arise either due to if 1) Production of superior hybrid substances in heterozygote is completely different from either of the homozygous products or due to 2) Greater buffering capacity in the heterozygote resulting from cumulative action of divergent alleles of stimulation of divergent alleles.

East in 1936 further elaborated this theory by proposing a series of alleles a_1,a_2,a_3,a_4 ----- of gradually increasing divergence in function. Thus a combination of more divergent alleles will exhibit higher heterosis than less divergent combinations. For example, combination of a_1, a_4 will exhibit higher heterosis as compared to combination as a_1, a_2, a_3 and a_4.

Overdominance has been reported in barley. In maize, available evidence suggests that if overdominance occurs, it is either infrequent in occurrence or small in magnitude. Dominance and overdominance hypothesis have some similarities and some dissimilarities.

3) EPISTASIS:

Epistasis refers to interaction between alleles of two or more different loci. It is also known as non-allelic interaction. The non-allelic interaction is of three type's viz. additive X additive, dominance X dominance and heterosis has positive association with the presence and magnitude of non allelic interaction. Epistasis, particularly that involves dominance effects (dominance X dominance) may contribute to heterosis. This has been observed in cotton and maize. Epistasis can be detected or estimated by various biometrical models.

Table 4: Comparison of Dominance and Overdominance Theories/ hypthesis of Heterosis

Particulars	**Dominance Hypothesis**	**Overdominance Hypothesis**

Similarities		
Effect of inbreeding	Inbreeding tends to decline in vigour	Also leads to decline in vigour
Out breeding	Out breeding restores vigour	Out breeding restores vigour
Parental diversity	Heterosis is associated with parental diversity.	Also associated with parental diversity
Control	Heterosis is controlled by genes	Also controlled by genes.
Difference		
Causes of heterosis	Heterosis results due to masking effect of dominant desirable allele or over harmful recessive alleles.	Heterosis results due to complementation between divergent alleles.

Effects and Use of Heterosis in Plant Breeding:

Manifestation (Effects) of Heterosis:

1) **Increased Yield:**

Heterosis is generally expressed as an increase in the yield of hybrid and may be measured in terms of grain, fruit, seed, leaf, tubers, etc.

2) Increased Reproductive Ability:

Hybrids exhibiting heterosis show an increase in fertility or reproductive ability.

3) Increase in Size and General Vigour:

The hybrids are generally more vigour, healthier and faster growing.

4) Better Quality:

In many cases, hybrid show improved quality. e.g., in Onion keeping quality is enhanced.

5) Earlier Flowering and Maturity:

Hybrids are earlier in flowering and maturity than the parents. e.g., tomato.

6) Greater Resistant to Disease and Pests:

Hybrid exhibits a greater resistance to insect of disease than parents.

7) Greater Adaptability:

Hybrids are more adapted to environmental changes than inbreds.

8) Faster Growth Rate:
Hybrids shows faster growth rate than their parents but the total size may be comparable to that of the parent.

9) Increase in Number of Plant Parts:
In some cases, there is an increase in the number of nodes, leaves and other plants parts, but the total plant size may not be larger.

Use of Heterosis in Plant Breeding:
Heterosis is exploited through the development of hybrid. It is commercially exploited in seed production of cross pollinated crops like bajara, cucurbit, jowar, maize, onion. It has been also used in some self- pollinated species such as brinjal, rice, tomato and wheat, etc.

Method of Plant Breeding in Cross Pollinated Plants – Hybridization

Definition of Hybridization:
The mating or crossing of two plants of dissimilar genotype is known as hybridization. In plants crossing is done by placing pollen grain from one genotype (male parent) on to the stigma of flower of another genotype (female parents).

The seed as well as the progeny resulting from the hybridization are known as hybrid or F_1. The progeny of F_1 obtained by selfing or intermating of F_1 and the subsequent generation are termed as segregating generation. Hybrid and synthetic variety have been highly successful in many cross – pollinated species. e.g., bajara, jowar and maize, and even in some self pollinated crops. e.g., rice, tomato, etc. In India almost all the recommended varieties of maize are either hybrid or composite varieties.

Definition of Hybrid:
The progeny of a cross between genetically different plants is called hybrid. In other word hybrid is F_1 generation of mating between genetically dissimilar plants. Most of the hybrid varieties are F_1 from two or more pure lines (*Lycopersicum esculentum*; tomato) or inbreds (maize; *Zea mays*).

An inbred is a nearly homozygous line obtained through continuous inbreeding of cross pollinated species. When F_1 generation from a cross between two or more pure lines inbreds or other genetically dissimilar population is used for commercial cultivation the resultant is called as hybrid variety. Hybrid varieties are most potent means for the exploitation of heterosis.

Types of Hybrid:

The commercially cultivated hybrids are of two types, i.e., A) Intraspecific hybrid and B) Interspecific hybrid

A) Intraspecific Hybrid:

It refers a hybrid between generally different genotypes of the same species. It is also known as Intervarietal hybrid. Intraspecific hybrids are always fertile. Based on type of cross, there are three types *viz.* (a. Single cross hybrid, b. Double cross hybrid and, c) Three way cross hybrid.

a) Single Cross Hybrid:

A cross between two inbreds or varieties is referred as single cross. e.g., (A X B) and the hybrid progeny obtained from a cross between two inbreds or varieties are referred as single cross hybrid.

In cross pollinated crops such hybrids are developed from a cross between two inbreds, whereas in self pollinated crops, they are developed from a cross between two homozygous varieties.

Merit features of Single Cross hybrid:

1. They are developed in both self and cross- pollinated crops, where heterosis is exploitable.
2. The total number of single crosses is n (n-1) /2 where 'n' is number of inbred line.
3. It is more common in some self –pollinated species than cross – pollinated species.
4. It is used for the development of double cross and three way cross hybrid.
5. It is also used to predict the performance of double cross hybrid.
6. It gives maximum degree of heterosis and produce uniform plant.

b) Double Cross Hybrid:

A cross between two single crosses is referred as double cross hybrid.

It is more commonly used in maize and sugarbeet and it involves for different inbred line viz., (A X B) X (C X D). The rows of female and male parents are planted in the ratio 4:1 in maize. The number of all possible double crosses among selected inbred is calculated by n (n-1) (n-2) (n-3) /8 where 'n' is number of inbreds involved.

c) Double Top Cross Hybrid:

It refers to the hybrid progeny between a single cross and an open pollinated variety. i.e. (A X B) X Open pollinated variety. It is used in maize.

(i) Top Cross: A cross between an inbred line and an open pollinated variety is known as top cross. It is also known as inbred variety cross and is used as testing the combining ability of inbreds and not for commercial hybrid seed production.

(ii) Multiple Crosses: A cross involving more than four inbred line is referred as multiple crosses.

(iii) Polycross: Open pollination of a group of selected genotypes in isolation from other compatible genotype to promote random mating among selected genotype. It was proposed by Tysdal *et. al.*(1942).

d) Three Way Cross Hybrid:

The hybrid progeny by crossing of gene and inbred lines is referred as three way cross hybrid. e.g. (A X B) X C. These hybrids sometimes used in maize, in which single cross is used as female and inbred as male and are planted in the ratio of 2:1. These hybrids produced seeds of normal shape and size but the main drawback is the low pollen production efficiency of the male inbred parent.

A) Interspecific Hybrid:

The F_1 progeny between two different progeny of the same genus is known as interspecific hybrid. It is also referred as intrageneric hybrid. These hybrids are rarely used for commercial cultivation because such hybrids are fertile only in few cases. In cotton interspecific hybrids between tetraploid cultivated sp. (*G. hirsutum* X *G. barbadense*) and diploid cultivated species (*G. arboreum* X *G. herbaceum*). *e.g.,* a tetraploid level: var. Laxmi, Surti, HB224, etc. At diploid level: DH-7, DH-9, and Pha 46, etc.

4.5 SUMMARY

Male sterile phenomenon and male sterility has important applications in the development of hybrids in different crops. All types of male sterility are used in crop improvement programmes. Besides applications male sterility has some disadvantages. It is very difficult to identify the male sterility line (When controlled by nuclear genes) before anthesis. In some cases GMS line becomes fertile under low or high temperature conditions. Moreover, sterile cytoplasm has adverse effects on yield components in some crops. Heterosis breeding has been commercially exploited in cross pollinated crops like, castor, cucurbits, maize, pearl millet, onion and sunflower. It has also been used in some self pollinated crops also like brinjal, rice and tomato etc. Due to heterosis it is possible to reconstitute the hybrid with same genotype, which is not possible in case of composite varieties and open pollinated varieties. But there are some demerits also associated with heterosis breeding. Fresh seed has to be required every year. The seed of hybrid is costlier than synthetics, composites and open pollinated varieties. Cultivation of hybrids requires more input (fertilizer, irrigation, plant protection, etc.) to exploit full potential. Production of hybrid requires more technical skill and areas as compared to synthetics, composites and open pollinated varieties. Thus by keeping in mind pro and cons of male sterility and heterosis one should give emphasis on optimum positive use of both phenomenon for the benefit of farming community in particular and whole mankind in wide spectrum.

4.6 GLOSSARY

Alleles: Alternative forms of a gene or DNA sequence occurring at the same locus on homologous chromosomes.

A line: The Male sterile line

Allosome: Sex chromosomes can be also called as allosome.

Autosome: Any chromosome other than the sex chromosomes.

B line: Isogenic line of a line with male fertility.

Carrier: A healthy person possessing a mutant gene in heterozygous form; also refers to a person with a balanced chromosomal translocation.

Chromosome: A structure within the nucleus composed of double stranded DNA bearing a linear array of genes that condenses and becomes visible at cell division.

Complete linkage: Inheritance patterns for two genes on the same chromosome when the observed frequency for crossover between the loci is zero.

Cytoplasmic Male Sterility: Pollen Sterility which is caused by cytoplasmic genes.

Cytoplasmic Genic Male Sterility: Pollen Sterility which is controlled by both cytoplasmic and nuclear genes.

Dioecious: If male and female are present on different plants then it is dioecious.

Diploid: It is that organism which has two sets of chromosomes.

Dominant: Trait expressed in people who are heterozygous for a particular gene.

Dominant Hypothesis: Heterosis due to superiority of dominant alleles over recessive alleles.

Doubled Haploids: Genetically pure plants that are developed through a special cross-breeding and chemical process. This process takes a fraction of the time of traditional inbreeding and provides improved parents for higher performing hybrids.

Economic heterosis: Superiority of F_1 Hybrid over the popular variety of a region; also called useful heterosis or standard heterosis.

F_1: Its full name is first filial generation. It is the first generation obtained by hybridization.

F_2: It is the second generation obtained on self fertilization of F_1.

F_3: It is the third generation obtained on selfing of F_2 hybrids.

Gamete: Egg or sperm. Cells formed after meiosis are called gametes.

Gene: The unit of inheritance composed of DNA. Genes are located in chromosomes.

Genetics: The field of biology that studies genes, genetic variation, and heredity in living organisms.

Genome: Total DNA carried by a gamete.

Genomics: The study of the genetic material in a chromosome set. This information gathered through genomic tools when used in conjunction with other technologies, helps researchers better understand which genes determine important characteristics and how genes work together.

Genotype: Genetic constitution of an individual person.

Genetic Male Sterility: Pollen sterility which is caused by nuclear genes.

Germplasm: A collection of genetic resources for an organism. The pioneer collection of maize genes used to develop hybrids, which is one of the most genetically diverse germplasm in the industry, is one example. These collections are critical resources for researchers who are committed to find genes that improve specific characteristics.

Hemizygote: Organisms having only one copy of a gene in diploid cells (males in human being are hemizygous for most X linked genes).

Heredity: The passing on of phenotypic traits from parents to their offsprings, either through sexual or asexual reproduction. Offspring cells or organisms are said to inherit the genetic information of their parents. It is also called inheritance.

Heterobeltiosis: Superiority of F_1 hybrid over the better parent for a particular character.

Heterosis: A term used in cross breeding to define when an organism has qualities that are superior to those of either parent.

Heterozygote: Person possessing different alleles at a particular locus on homologous chromosomes.

Homologous: Chromosomes that pair at meiosis chromosomes and contain the same set of gene loci. Person having two identical alleles for a given gene at a particular locus on homologous chromosomes; both alleles at corresponding loci are identical.

Hybrid: Hybrid is the organism borne by crossing parents of different genotype.

Hybrid Variety: The F_1 population which is used for commercial cultivation. Hybrid varieties are of two types, *viz*., intraspecific hybrid and interspecific hybrid.

Karyotype: An array of all the chromosomes found in a cell of an individual. Typically the chromosomes are stained to reveal size, banding pattern or other distinguishing features to enable the identification of any abnormalities.

Linkage: Genes that are inherited together on the same chromosome.

Locus: Site of a specific gene or DNA sequence on a chromosome.

Luxuriance: Superiority of F_1 over its parents in vegetative growth but not in yield and adaptation.

Male Sterility: A condition in which either pollen is absent or non-functional in flowering plants.

Male Gametocide: Chemicals which are used for induction of male sterility.

Mid Parent heterosis: Heterosis over the mean value of both the parents; also called mean heterosis.

Multiple cross: A cross involving more than four inbred lines; also known as composite cross.

Polycross: Open pollination of a group of selected genotypes in isolation from other compatible genotypes to promote random mating among selected genotypes.

R line: A line which restores fertility when crossed with cytoplasmic genic male sterile line.

4.7 SELF- ASSESSMENT QUESTIONS

4.7.1 Fill in the blank

a) The pollen sterility which is caused by nuclear genes is referred to as……(Genetic Male Sterility)

b) The……..male sterility is caused by cytoplasmic genes or plasma genes. (Cytoplasmic)

c) Pollen sterility which is caused by both nuclear and plasma genes is known as…….. (Cytoplasmic genic male sterility)

d) Cytoplasmic sterility is maintained by crossing A line with ……..(B line)
e) Mutagen induced male sterility is ……(Heritable)
f) Male sterility induced by gametocide is…….(Non -heritable)
g) Superiority of F_1 in fitness and vigour over its parent is called……(Heterosis)
h) Luxuriance is also refered to as……..(Pseudoheterosis)
i) Masking effect of one allele over the other on the same locus is called……(Dominance)
j) The term over dominance was coined by……..in…….(Hull 1945)

4.7.2 Very Short Answer Type Questions

a) Define male sterility.
b) Define heterosis,
c) What is pollen sterility?
d) Is hybrid corn sterile?
e) Who first reported male sterility in flowering plants?
f) What do you mean by luxuriance?
g) What is heterobeltiosis ?
h) What is hybrid vigour in Biology?
i) Who first reported CMS in crop plants?
j) Who first reported CGMS in crop plants?
k) What is A line?
l) What is B line?
m) What is R line?
n) What is gynodioecy?

4.7.3 Short Answer Type Questions

a) What are basic differences between self- incompatibility and male sterility?
b) Compare genetic male sterility (GMS) and Cytoplasmic Genic Male Sterility (CGMS).
c) What are objections to dominance theory of heterosis?
d) What are main features of hybrids?
e) Compare dominance and over dominance theories of heterosis.
f) What are demerits of hybrid varieties?
g) What is top cross?
h) What is double top cross?
i) What is multiple cross?
j) How the performance of a double cross hybrid is predicted?
k) What is pseudo heterosis?
l) How do you calculate heterosis?

4.7.4 Multiple choice questions:

1. Male sterility in flowering plants was first reported by:
(a) Kolreuter (1763) (b) Duvick (1966)
(c) Allard (1960) (d) Frankel and Galun (1977)

2. The term heterosis was coined by:
(a) East (1914) (b) East (1908)
(c) Hull (1945) (d) Devenport(1908)

3. Overdominance hypothesis is also called as:
(a) Super dominance (b) Single gene heterosis
(c) Stimulation of heterozygotes (d) All of the above

4. Heterosis over the best commercial variety is referred to as:
(a) Mean heterosis (b) Heterobeltiosis
(c) Useful heterosis (d) None of the above

5. Potato belongs to family:
(a) Asteraceae (b) Convolvulaceae
(c) Solanaceae (d) Euphorbiaceae

6. A plant breeder is interested to control pollination to:
(a) Prevent cross pollination (b) Prevent self pollination
(c) None of these (d) Both of these

7. Highest uniformity is observed in a:
(a) Single cross (b) Three way cross
(c) Double cross (d) Multiple cross

8. Highest adoptability is observed in:
(a) Single cross (b) Double cross
(c) Three way cross (d) Multiple cross

9. Heterobeltiosis is estimated over the:
(a) Mid parent (b) Better parent
(c) Popular Variety (d) Popular Hybri

10. Out breeding leads to reduction in:
(a) Homozygosity (b) Heterozygosity
(c) Population mean (d) All of the above

4.7.4 Answer Key:

1. (a), 2.(a), 3.(d), 4.(b,) 5.(c), 6.(d), 7.(a), 8.(d), 9.(b), 10.(a)

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4.9 TERMINAL QUESTIONS

1- Describe the importance of male sterility in plant breeding.
2- What kind of male sterility can be used in crops where seed is not a commercial product?
3- Discuss briefly the various sources of male sterility in plants.

4- Describe genetic, cytoplasmic and cytoplasmic genetic male sterility systems in crop plants. Explain their utility and cite suitable examples.
5- Explain briefly the various features of heterosis in crop plants.
6- Give the genetic and physiological basis of heterosis.
7- Discuss various factors affecting the magnitude of heterosis in crop plants.
8- Enumerate various methods of fixation of heterosis in crop plants with examples.

UNIT-5 APOMIXIS: APPLICATION IN PLANT BREEDING

5.1 OBJECTIVES

After reading this unit students will be able-

- To understand the basics of apomixis
- To know the types of apomixis
- To workout the applications of apomixis in plant breeding

5.2 INTRODUCTION

First coined by Hans Winkler in 1906, the term apomixis refers to asexual plant reproduction that occurs without any form of fertilization. Unlike asexual propagation from leaves or cuttings, apomixis is categorized by its lack of outside intervention, such as replacement of seeds by plantlets or flowers by bulbils. The process of apomixis gives rise the plants that are genetically identical to the parent plants. Apomixis refers to the occurrence of an asexual reproductive process in place of normal sexual processes involving reduction division and fertilization.

Apomixis has received increased research emphasis in past 30 years due to discoveries of partially apomictic (facultative) plants. In cultivated species, discoveries of sexual plants in apomictic species, new information on genetic control and a broader awareness of the impact that apomixes could have on cultivar development.

Apomixis could have a major impact on seed-propagated food, forage and fibre production around the world. It would especially be beneficial in the major annual grains such as wheat, rice and soybean where hybrid vigour is present but systems for commercially producing hybrids may not be available and economical. In crops such as maize, sorghum and pearl millet, commercial hybrid production systems are available but apomixes could have a major impact by simplifying hybrid seed production and by making hybrids readily available and affordable in developing countries especially in India.

It differs from amphimixis which involves union of male and female gametes for development of seed.

TABLE: 1 Difference between Apomixis and Amphimixis

S.No.	Attributes	Apomixis	Amphimixis
1.	Sexual fusion or union of male and female gametes	Does not involve	Involves
2.	Gene combination or gene flow	Not possible	Possible
3.	Segregation	Does not occur	Occurs
4.	Rapid development of inbred lines	Possible	Not possible
5.	Conservation of heterosis	Possible	Not possible
6.	Type of reproduction	Asexual	Sexual
7.	Frequency of occurrence of Plants	Low	High

In other words, apomixis is a type of reproduction in which sexual organs of related structures take part but seeds are formed without union of gametes. Seeds formed in this way are of vegetative origin.

When apomixis is the only method of reproduction in a plant species, it is known as obligate apomixis. On the other hand, if gametic and apomictic reproductions occur in the same plant, it is known as facultative apomixis.

5.3 APOMIXIS

There are four types of apomixes on the basis of cell involved *viz*., 1- parthenogenesis 2- apogamy, 3- apospory and 4- adventives embryony. These are briefly discussed as follows and comparative study is displayed in Table: 2

1. **Parthenogenesis:** Parthenogenesis refers to development of embryo from the egg cell without fertilization. It is of two types- (1) haploid and (2) diploid. When the embryo develops from a haploid egg cell, it is known as haploid parthenogenesis. The plants which develop from such embryos are haploid and sterile. Haploid parthenogenesis is found in *Solanum nigrum*. Sometimes, embryo sac develops without reduction division. Such parthenogenesis is known as diploid parthenogenesis and has been reported in grasses like Taraxacum. In plant species like tobacco and rice pollen grains may be induced to develop into embryos. This development of embryos from pollens or anthers is termed as androgenesis.

There are several causes of parthenogenesis. The main causes include:-

(1) Inability of the pollen tube to discharge the contents inside the embryo sac.
(2) Insufficient attraction between male and female gametes.
(3) Early degeneration of the sperm.
(4) Very long style.
(5) Schlerenchymatous style.
(6) Short pollen tube.
(7) Slow rate of pollen tube growth.
(8) Stimulation of pollination in the absence of pollen tube.
(9) Incompatibility.

Parthenogenesis can be artificially induced by four main ways :

(1) By the stimulation of widely related pollen or foreign pollen.
(2) By low temperature.
(3) By pollinating with X-ray irradiated pollens.
(4) By treatment with certain chemicals. The chemicals help in inducing parthenogenetic development of egg cell.

(2) Apogamy: the origin of embryo from either synergids or antipodal cells of the embryo sac is called apogamy. It is of two types-: (1) haploid apogamy and (2) diploid apogamy. The synergids or antipodal cells may be haploid or diploid. If embryo develops from haploid synergids or antipodal cells, it is known as haploid apogamy when the embryo develops from diploid synergids

or antipodal cells; it is called as diploid apogamy. Diploid apogamy has been reported in *Allium*, *Iris* and many other species.

(3) Apospory: In apospory, first diploid cell of ovule lying outside the embryo sac develops into another embryo sac without reduction. The embryo then develops directly from the diploid egg cell without fertilization. Apospory is of two types; (1) generative apospory and (2) somatic apospory. In generative or haploid apospory the embryo develop from one of the megaspores (n), since it cannot regenerate as it is haploid and fertilization fails the process giving rise to non recurrent apmicts as in *Parthenium.* Somatic apospory is the formation of complete embryo from the somatic cell or when the embryo originates from the embryo sac that has developed from the cell of either nucellus or integument. This is found in *Malus, Crepis Poa* and many other crop species.

(4) Adventive embryony: The development of embryo directly from the diploid cells of ovule lying outside the embryo sac belonging to either nucellus or integuments is referred to as adventives embryony. There is no production of another embryo sac like apospory. This is a type of sporophytic budding which is very common in *Citrus* and mango.

Apomixis is also classified as recurrent (2n) and non recurrent (n). In recurrent apomixis the embryo sac develops from diploid cells. There is no reduction in the chromosome number and all the cells of embryo sac are diploid. This process is repeated from generation to generation and hence it is called recurrent apomixis. It includes: (1) diploid parthenogenesis, (2) diploid apogamy and, (3) apospory.

There are two types of apomixis on the basis of occurrence:

(a) Recurrent apomixes: In recurrent apomixis, both the egg cell and embryo are diploid and the embryo sac is developed from the megaspore mother cell. In this type of apomixis an embryo sac develops from the megaspore mother cell where meiosis is disturbed or from some adjoining cell. Consequently, the egg cell is also diploid. The embryo subsequently develops directly from the diploid egg cell without fertilization. Somatic apospory, diploid parthenogenesis and diploid apogamy are recurrent apomixis. However, diploid parthenogenesis/ aposporogamy occur only in aposporic (somatic) embryo sacs. Therefore, it is the somatic or diploid apospory that constitutes the recurrent apomixis. Such apomixis occurs in some species of *Crepis* and *Taraxacum,* without the stimulus of pollination.

(b) **Non-recurrent Apomixis** : In non-recurrent apomixis, both the egg-cell and embryo are haploid and embryo is developed directly from an egg cell without fertilization. Since an egg cell is haploid, the resulting embryo will also be haploid. Haploid parthenogenesis and haploid apogamy and androgamy fall in this category. Such types of apomixis are of rare occurrence. They do not perpetuate and are primarily of genetic interest in Corn.

There are two types of apomixis on the basis of frequency:

(1) **Obligate apomixis:** This apomixis is an asexual means of reproduction. Apomixis is found in many crop species. This apomixis is termed as obligate apomixis.

(2) **Facultative apomixis**: Facultative apomixis means that apomixis does not always occur, *i.e.* sexual reproduction can also happen. It appears likely that all apomixis in plants is facultative.

TABLE 2: Comparative analysis of classification of Apomixis

S.No.	Basis of Classification	Types of Apomixis	Specific Characteristic
1.	Cell involved	Parthenogenesis	Embryo develops from egg cell
		Apogamy	Embryo develops from either synergids and antipodal cells
		Apospory	Embryo develops from the embryo sac which has developed from the cell of archisporium/nucellus or integument.
		Adventive Embryony	Embryo develops directly from the diploid cell of either nucellus or integument.
2.	Occurrence	Recurrent apomixis	Embryo sac has diploid cells and embryo develops from the diploid cells.
		Non-recurrent apomixis	Embryo sac consists of haploid cells and embryo develops from haploid cell.
3.	Frequency	Obligate apomixis	The reproduction occurs by apomictic means only
		Facultative apomixis	The reproduction occurs by both sexual and apomictic means.

In nutshell we can say apomixis, an asexual method of reproduction through the seed, provides unique opportunities for developing superior cultivars in the future. Apomixis occurs at low levels in some cultivated species and can be found in closely related species of many cultivated crops. Transfer of apomixis to important crops would make possible development of true breeding hybrids and commercial production of hybrids without a need for cytoplasmic male sterility and high cost, labour intensive processes. Obligately apomictic hybrids would breed true regardless of heterozygosity. It could also provide an efficient method for incorporating genes into new genotypes. Superior obligate apomictic genotypes would be ready for performance testing without a need for progeny testing to determine genetic stability. The commercial hybrid production process would be simplified with apomictic hybrids. Several apomixis mechanisms have been shown to be genetically controlled and therefore subject to genetic manipulation in plant breeding programs.

Genetic Basis for Apomixis: Scientists for over a century have recognized that apomixis is transmitted from parent to progeny. Even Mendel attempted early genetic analysis of crossings between sexual mother plants and apomicts. A lack of inheritance data needed for critical analysis has posed problems in understanding apomixis.

Theories of inheritance with apomicts have evolved over time. Early theories ware dominated with ideas of polyploidy and hybridity. To reach these conclusions researchers studied the nature and behavior of apomicts and observed that most apomictic plants were polyploids and, cytologically resembling species hybrids. Physiological factors and hormones were thought to be direct cause of apomixis. Due to the complexity of the apomictic system and lack of inheritance data, it has been difficult to develop a single genetic explanation for apomixis. Several theories are currently being debated and can be divided in to theories regarding apospory and diplospory.

5.4 APPLICATIONS OF APOMIXIS IN PLANT BREEDING

Apomixis has several useful applications in plant breeding. The important applications are given below:

1. **Rapid production of pure lines:** Apomixis is an effective means for rapid production of pure lines. Haploid parthenogenesis gives rise to haploid plants which upon colchicines treatment will produce diploid pure lines that can be used in plant breeding program.
2. **Maintenance of superior genotypes:** A superior plant type which produces seed by apomictic means will usually breed true for the characteristics of mother plant. Thus apomixis is useful in maintaining the characteristics of mother plant from generation to generation.
3. **Conservation of heterosis:** In some cases, hybrid vigour may be conserved for many generations by using recurrent apomixis.
4. **Impact on seed industry:** Apomixis would no doubt have a positive impact in seed industry. The way commercial cultivar are produced and increased, production practices would be radically changed and at the same time greatly simplified. The need to maintain and increase parental lines and the need to be concerned about isolation to prevent out crossing would be eliminated. The advent of individual hybrid plant selection, in a world of sexual crops, where improvement has therefore been on family based strategies, will enable scientists to create totally new breeding schemes, much more related to those used in clonal crops.

The two sexual processes, self and cross fertilization, followed by segregation, tend to alter the genetic composition of plants reproduced through amphimixis. Inbreeding and uncontrolled out breeding also tend to break heterozygote superiority in such plants. On the contrary, apomict tends to conserve the genetic structure of their carriers. They are also capable of maintaining heterozygote advantages generation after generation. Therefore such a mechanism might offer a great advantage in Plant Breeding where genetic uniformity maintained over generation for both homozygosity (in selfing varieties) and, heterozygosity (in hybrids of both in breeders and out breeders) is the choicest goal. Additionally, apomixes may also affect an efficient exploitation of maternal influence, if any, reflecting in the resultant progenies, early or delayed because it causes

the perpetuation of only maternal individuals and maternal properties due to prohibition of fertilization. Maternal effects are most common in horticultural crops, particularly fruit species.

Thus in short the benefits of apomixes, so far as their utility in Plant Breeding is concerned are:

1. Rapid multiplication of genetically uniform individuals can be achieved without the risk of segregation
2. Heterosis or hybrid vigour can permanently be fixed in crop plants, thus no problem for recurring seed production of F_1 hybrids.
3. Efficient exploitation of maternal effect, if present, is possible from generation to generation.
4. Homozygous inbred lines, as in corn, can be rapidly developed as they produce sectors of diploid tissue and occasional fertile gametes and seeds.

5.5 SUMMARY

This unit intends to introduce many ways to identify apomixes, its incidence in flowering plants and the present state of knowledge in the field. The characterization of the phenomenon will be improved with the advent of large apomictic hybrid analysis with practical results. Still this technique has lots of prospects like ease of multiplying and maintaining elite hybrid genotypes, eases of producing high quality pure seeds without isolation. Besides this there are also possibilities for selection of more closely adopted diverse genotypes as expected for fixation of hybrid vigor and lower the cost of hybrid seed production. Documentation method would need to be refined and precise because of proliferation of cultivars in the markers, some with only small differences.

Modern molecular genetics are being used as a tool to physiological or biochemical pathways responsible for changing the role of specific cells giving rise to apomictic cells. Currently, apomeiosis loci have been identified. However recombination around these loci is suppressed, making map based cloning difficult. The degree of complexity of these loci is unknown.

Although the mechanism of apomictic species has been studied in relatively few species, there are several proposed models for its genetic control. Theories of inheritance range from a single apomixis locus or linkage group common to all apomicts to independent, random mutations at various reproductive loci. The truth about inheritance of apomixes appears to lie somewhere between two extremes. Whatever the underlying mechanism, the genes that are affected by these loci are likely to play important roles in sexual development and understanding of apomixes.

5.6 GLOSSARY

Androgenesis-A haploid embryo develops from the haploid sperm nucleus.

Anterior - Front, away from the axis.

Anther - The pollen bearing part of a stamen.

Anther cap - The cap like structure which terminates the column and covers the pollinia.

Anthesis - The period of flowering.

Apogamy- Development of sporophyte directly from gametophyte, without fusion of gametes, such sporophytes have the same chromosome number as the gametophyte from which they have been derived.
Apomeiosis - Without meiosis"; usually meaning the production of a meiotically unreduced gametophyte
Apomicts - A plant that arises from apomixis.
Apomixis - The production of seeds without the union of sex cells; this is actually a process of vegetative reproduction.
Asexual propagation - Propagation by vegetative means; for instance by division, aerial growths or meristem culture.
Autogamy - The process of self pollination.
Basal - Arising from the base; often said of the point where an inflorescence arises.
Beak - A point; often used for the apical part of the anther (see also rostrum).
Bilocular - With two cavities or locules.
Bisexual - Both male and female sexes present.
Cleistogamy - The process of self pollination occurring without the flowers opening.
Clinandrium - The apical margins of the column or the cavity where the anther fits.
Clonal - Pertaining to a clone, a group of plants propagated vegetatively from one plant.
Clone - A group of plants propagated vegetatively from one plant (usually a superior horticultural form); all members of a clone are genetically identical.
Connective - The sterile portion of the anther between the two anther cells.
Co-pollinator - Where more than one kind of pollinator acts effectively in the pollination of a plant.
Cross fertilisation - Fertilisation by pollen from another flower.
Cross pollination - Transfer of pollen from flower to flower.
Cultivar - A horticultural variety of a plant or crop.
Cuniculus - An extra floral nectary found on the pedicel or ovary.
Cyme - An inflorescence where the branches are opposite.
Cymose - Is the form of a cyme with a flower at the end of each branch *i.e.* a determinate inflorescence.
Cytomixis- A process of nuclear fusion that occurs during pollen meiosis.
Dehiscent - Splitting or opening when mature.
Determinate – It is a growth or inflorescence when it has an extension limit. The apical bud is consumed in the formation of a flower.
Diandrous - With two anthers.
Dichotomous - Forking regularly into two equal branches or parts.
Dicotyledon - Any Angiosperm which has two seed leaves and reticulate venation in the leaves.
Dimorphic - Existing in two different forms.
Dimorphism - The non-flowering plants are strikingly different to that of flowering plants.
Distal - Away from the base towards the apex.

Distichous - In two rows; usually applied to the arrangement of leaves or flowers.
Diurnal - During the day, as describing flowers that only open in the day.
Dormancy - A physical or physiological condition that prevents growth or germination even though external factors are favourable.
Dormant - The state of a plant when growth has ceased for the year and other activities in the plant have slowed down, usually during winter.
Down curved - The apex curves downwards but is not recurved back on itself.
Downy - Covered with soft hairs.
Dropper - A term used for the short root which bears a replacement tuber (also known as a sinker).
Duplicate - Leaves which are folded once along the centre, the two halves being flat.
Ecology - The study of the interaction of plants and animals with their natural environment.
Eglandular - Hairs that do not have a swollen gland-like structure at the apex; without glands.
Endemic - Restricted to a particular country, region or area.
Endosperm - Tissue rich in nutrients which surrounds the embryo in most seeds; orchid seeds lack endosperm.
Ephemeral - Short-lived; in flowers referring to those which last a few hours or less. Ephemeral Short lived or of short duration.
Epilith - Growing on rocks.
Epiphyte - A plant growing on or attached to another plant but not drawing nourishment from it and therefore not parasitic.
Epiphytic - Growing on or attached to another plant but not drawing nourishment from it and therefore not parasitic.
Epiphytosis - A condition of decline in trees attributed to having too many epiphytes on their trunk and branches.
Equitant - Laterally flattened leaves arranged in two ranks that overlap at the base.
Excrescences - Warty outgrowths that often secrete water; also used for structures on the labellum margins of *Microtis* that resemble clusters of cells.
Exotic - A plant introduced from overseas.
Exerted - Protruding beyond the surrounding parts.
Extra floral - Occurring outside of a flower; as in extra-floral nectaries.
Eye - A horticultural term used for a viable vegetative bud.
Falcate - Sickle shaped.
Family - A taxonomic group of related genera.
Fertile - Mature plant capable of flowering and producing seed.
Fertile bract - A bract which subtends a pedicel (compare with sterile bract).
Fertilisation - The act of union of the male gametes (from the pollen) with the egg cells in the ovules.
Forked - Divided into two equal segments.

Four lobed - In few orchids that have well developed lateral lobes the mid lobe is deeply divided, the whole structure appearing as if it is four lobed.
Free - Not joined to any other part except at the base.
Fruit - The seed bearing organ developed after fertilisation.
Furrowed - Grooved longitudinally.
Fusiform - Spindle shaped; widest in the middle and tapered to each end.
Gammate - Resembling an upside down capital L; used for calli shape in some genera related to *Caladenia*.
Gamopetalous - With the petals fused.
Gamosepalous - With the sepals fused.
Genera - A taxonomic category ranking below a family and above a species and generally consisting of a group of species exhibiting similar characteristics.
Genus - A taxonomic group of closely related species.
Geophyte - A plant growing in the ground.
Geophytic - A plant growing in the ground.
Germination - The active growth of an embryo resulting in the development of a young plant.
Gibbosity - Something that bulges out or is protuberant or projects from a form, plane or margin.
Gland - A secreting surface or structure; loosely used for any protuberance or appendage having the appearance of such an organ, such as calli.
Gynandrium - Another term for the column.
Gynoecium - The female parts of a flower.
Habit - The general appearance of a plant.
Habitat - The environment in which a plant grows.
Hamulus - A specialised stipe which is the recurved apex of the rostellum; found in species of *Prasophyllum*.
Haploid - With one set of chromosomes.
Head - An inflorescence with the flowers in a tight cluster; for example, *Aster*.
Hemipollinarium - Half of a pollinarium which is removed as a unit; this structure occurs in orchids which have two viscidia.
Herb - A plant which produces a fleshy rather than a woody stem.
Herbaceous - A perennial plant which dies down each year after flowering (see also deciduous).
Herbarium - A botanical collection of dried and pressed plant specimens.
Hermaphrodite - Having male and female parts on a flower.
Hybrid - The progeny of a cross between two species, cultivars or other hybrids.
Hybridisation - The act of crossing flowers to produce hybrids.
Imbricate - Overlapping like fish scales.
Incised - Deeply and irregularly cut.
Incumbent - Said of an anther which bends forwards during the development of the flower.
Incurved - Curved inward.
Indehiscent - Not splitting open at maturity.

Indeterminate - Said of a growth or inflorescence when it has no apparent extension limit.
Inflorescence - The flowering structure of a plant.
Labellum - A lip; in orchids and gingers the highly modified ventral petal that is primarily involved in pollination.
Labellum lamina - The expanded part of the labellum, the broad middle part of the labellum.
Lacerate - Appearing as if irregularly cut or torn.
Medial - Relating to the middle, *i.e.,* a medial ridge is in the middle of the labellum.
Medium - The potting mix in which an orchid is grown, or the mixture on which seeds are raised.
Membranous - Like a membrane; thin-textured.
Mentum - A chin-like extension at the base of a flower consisting of the column-foot fused with
Mitra - Column hood formed by the sterile stamen and/or filament.
Monad - A single pollen grain unattached to others; as opposed to a tetrad.
Monandrous - With one anther.
Moniliform - Having a chain like series of bumps, swellings or joints that resemble beads on a string.
Monocarpic - Flowering and fruiting only once before dying.
Monocotyledon - Any Angiosperm which has one seed leaf and parallel venation in the leaves.
Monomorphic - The leaves on the flowering plant are the same form and arrangement as those on the non-flowering plant.
Monophyletic - Having a single ancestor.
Monopodial - A stem with a single main axis which grows forward at the tip.
Naked - A term used for pollinia which lack any supporting structures such as stipes or caudicles.
Non-resupinate - An orchid flower which has the dorsal sepal below the lateral sepals and the labellum above the column.
Offset - A growth arising from the base of a plant and producing roots while still attached.
Ovary - The part of the gynoecium which encloses the ovules and after fertilisation develops into the fruit.
Ovule - The small structure within the ovary which becomes a seed after fertilisation.
Paniculate - Arranged in a panicle.
Parthenogenesis: A haploid embryo developing from the haploid egg.
Perianth - A collective term for the petals and sepals of a flower, in orchids this does not include the labellum.
Perianth segments - A collective term for the petals and sepals of a flower, in orchids this does not include the labellum.
Placenta - The place in the ovaries that bears the seeds.
Placentation - The arrangement of the ovules in the ovary.
Pollen - The one celled male spores that are borne in the anther.
Pollen scraper - A term used for a rigid part of the rostellum which directs insect borne pollinia into the stigma.
Pollinarium - The whole male structure as moved by an insect during pollination.

Pollination - The transference of pollens from the anther to the stigma of a flower.
Pollinator - An insect that carries pollen grains from one flower to another.
Pollinia - Coherent or incoherent structures consisting wholly of aggregated pollen grains.
Protoembryo - A term used for orchid embryos because they lack any differentiation into tissues.
Proximal - Situated near the point of attachment.
Pseudogamy-Pollination serves as stimulus for embryo development but the egg and sperm nuclei do not fuse. Fusion of the polar nuclei with one of the sperm nuclei may occur to produce endosperm.
Pseudopollen – Starch rich cells produced on the labellum of some orchids and which are collected during pollination.
Raceme - A simple unbranched inflorescence with stalked flowers. It is indeterminate with indefinite axis.
Recumbent - Said of anthers which are strongly bent so that the apex is below the level of the base.
Resupinate - Inverted. In the Orchidaceae, many of the flowers are twisted through 180 degrees, so that the position of the upper and lower petals is reversed. An orchid flower which has the dorsal sepal above the lateral sepals and the labellum below the column.
Reticulate - With veins that interconnect like a net.
Retinaculum - Another term for a viscidium.
Retrorse - Pointed strongly backwards towards the base.
Retuse - The apex rounded and with a shallow notch.
Revolute - With the margins rolled back.
Rachis - The main axis of a compound leaf or an inflorescence (to which the pedicels or petioles are attached).
Scale - A dry flattened, papery body; sometimes also used as a term for a rudimentary leaf.
Scape - The peduncle and rachis of an inflorescence.
Seed - A mature ovule containing an embryo and capable of germinating.
Seed coat - The protective covering of a seed; also called testa.
Seedling - A young plant raised from seed which has not yet flowered.
Semigamy-The haploid sperm nucleus enters the egg but does not fuse with the haploid egg nucleus. Each nucleus divides independently creating a haploid embryo that contains sectors of male and female origin.
Shoot - A horticultural term used by growers for a new growth.
Species - A taxonomic group of closely related plants all with similar basic features.
Striae - A thin line or band, especially one of several that are parallel or close together.
Stylar canal - A channel from the style into the ovary through which the pollen tubes grow.
Style - The slender part of the pistil which connects the stigma with the ovary; in orchids the style forms an indiscernible part of the column.
Subcoriaceous - Somewhat leathery.
Subgenus - A subdivision of a genus.

Suborbicular - Almost circular.
Subsessile - Very shortly stalked.
Subsimilar - Somewhat similar.
Subtend - To support another structure or organ.
Subterranean - Below ground; plants which spend their life cycle below ground.
Subulate – Awl shaped; with a stiff point that tapers from base to apex.
Succulent - Fleshy or juicy.
Sucker - A shoot arising from the roots or the trunk below ground level.
Sympatric - Growing together.
Sympodial - A growth habit whereby each stem has limited growth and new shoots arise from the base of previous ones.
Syndrome - The complex of floral features which suggests adaptation to a particular pollinator group.
Taxon - A term used to describe any taxonomic group, for example, genus and species.
Taxonomy - The classification and naming of plants or animals.
Tetrad - A unit of four pollen grains.
Trichome - A hair like growth.
Trifid - The apex divided into three lobes.
Trigonous - Distinctly three cornered and triangular in cross section.
Trilobed - With three lobes.
Tripartite - Composed of or divided into three parts.
Truncate - As if cut off square at the apex.
Tuber - A thickened underground storage organ derived from a root.
Umbel - An inflorescence where the flowers radiate from a single point, as in *Centella asiatica* (Hydrocotyl) and *Bupleurum*.
Unisexual - Of one sex only; staminate (male) or pistillate (female).
Variegated - Where the basic colour of a leaf or petal is broken by areas of another colour, usually white, pale green or yellow.
Variety - A taxonomic subgroup within a species used to differentiate variable populations..
Vegetation - The whole plant communities of an area.
Vegetative - Asexual development or propagation.
Ventral - On the lower side.
Verrucose - Covered with warts or wartlike projections.
Viable - Alive and able to germinate, as of seeds.
Viscid - Very sticky or glutinous.
Viscin - Clear elastic threads found in pollinia.
Viscous - Very sticky.
Whorl - Three or more segments (of leaves, flowers) in a circle at a node.
Winged - Having flat projections longitudinally along an axis.

Zygomorphic - Asymmetrical and irregular. A flower which cannot be divided equally in more than one plane.

5.7 SELF- ASSESSMENT QUESTIONS

5.7.1 Fill in the blank:

a) ……is a process of nuclear fusion that occurs during pollen meiosis.
b) The development of embryo from the egg cell without fertilization is known as……..
c) The act of crossing flowers to produce hybrids is known as……..
d) Pollination is best defined as…….
e) Pollination which occurs in closed flower is called……...
f) Pollination is characteristic of ……
g) The term apomixis in 1906 was coined by…….
h) Offsprings receive valuable qualities from both parents in…….
i) Functional megaspore in an angiosperm develops into…….
j) Haploid parthenogenesis is found in.....
k) The male reproductive parts of a flower, the stamens, are collectively known as…....……
l) The other name for gynoecium is .....

5.7.1 Answer key: a. cytomixis, b. parthenogenesis, c. hybridization, d. transfer of pollen from anther to stigma, e. cleistogamy, f. angiosperms, g. Hans Winkler, h. cross pollination, i. embryo sac, j. *Solanum nigrum*, k. androecium, l. pistil

5.7.2 Very Short Answer Type Questions:

a) Define apomixis with live examples.
b) What is basic difference between apospory and apogamy?
c) What is apomictic hybrid?
d) What are the causes of apomixis?
e) How does apomixis differ from automixis?

5.7.3 Short Answer Type Questions:

1. What is obligate apomixis?
2. What are the causes of apomixis?
3. What is Apomixis with example?
4. How Apomictic seeds are produced?
5. What are the advantages of apomixis?
6. Why are some seeds referred to as apomictic seeds?
7. What are the types of apomixis?
8. What is facultative apomixes?
9. What is sporophytic apomixis?
10. What are the basic differences between recurrent and non recurrent apomixes?

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5.10 TERMINAL QUESTIONS

5.10.1-Long Answer Type Questions:

1- Describe different types of apomixes.

2-What are basic differences between different types of apomixis.

3- Illustrate mechanism of apomixes in plants.

4- Write elaborately comparative analysis of classification of Apomixis.

5- Describe the applications of apomixes in plant breeding

6- Differentiate between Apomixis and Amphimixis.

7- Embryo sac of some apomictic species appears normal but contains diploid cells. Suggest a suitable explanation for the condition.

8- What do you know about genetic basis of apomixis.

UNIT-6- RESISTANCE BREEDING FOR TEMPERATURE, FROST, SALT AND ACID TOLERANCE, LODGING AND DISEASES

6.1 OBJECTIVES

After reading this unit students will be able:

- To study basics of resistance breeding
- To study types of resistance breeding
- To understand the applications of resistance breeding

6.2 INTRODUCTION

Stress refers to adverse conditions for crop growth and production imposed by either environmental factors or biological factors. The stress is of two types, *viz.* (1) Abiotic and (2) Botic. The stress which is caused by environmental factors or non biological factors is referred to as abiotic stress. Abiotic stress is generally caused by factors like temperature, light, deficiency or excess of nutrition, moisture, abnormal soil conditions such as salinity, alkalinity and acidity and lodging. The stress that is caused by biological agents or factors such as diseases, insects or parasitic weeds is known as biotic stress.

6.3 RESISTANCE BREEDING FOR TEMPERATURE

A temperature higher or lower than the optimum range of temperatures for normal crop growth constitutes temperature stress: (i) Heat Stress (ii) Chilling stress and (iii) Freezing stress.

Heat stress is generated by temperature higher than the optimal range. Heat affects (i) cell and tissue survival (ii) growth and development and (iii) physiological parameters (e.g., respiration, photosynthesis, photosynthate translocation, protein denaturation, membrane composition and stability and, production of heat shock proteins, HSP).

Heat resistance is the ability of some genotypes to perform better than others under heat stress. Heat resistance mechanisms may be grouped as (i) heat avoidance and (ii) heat tolerance. Heat avoidance is the ability of a genotype to dissipate the radiation energy primarily by transpiration and due to reflective properties of leaves. Heat tolerance, on the other hand, is ability of some genotypes to perform better than others at elevated temperatures; it may involve (i) membrane stability (ii) reduced heat sensitivity of photo-system II (iii) photosynthate translocation (iv) stem reserve mobilization (v) and osmo-regulation. Expression of heat tolerance is highly dependent on heat hardening, i.e. a brief (20 sec to 6 hr at 50^0 C) exposure to a high temperature leading to improved resistance to heat later. Heat hardening is most likely due to HSP.

Heat tolerance is usually under polygenic control; both additive and dominance gene effects are important. In most crops, heat tolerance is available within the breeding germplasm. When required, such lines may be found in collections from margins of geographical distribution of the concerned crop. Selection can be carried out in the concerned region (or at a different location), or in green houses and growth chambers; some assays, e.g., membrane stability test can be done in test tubes.

Selection for heat tolerance may be based on the following: (i) growth under heat stress (ii) yield under heat stress (iii) flower, fruit, seed etc. formation, including pollen fertility (iv) seed germination under heat stress (v) recovery after heat stress (vi) sensitivity of the photosynthetic process and (vii) membrane stability after heat shock. It is a fair index of performance under stress and quick to determine.

Chilling stress is imposed by temperatures above zero but below the optimal range. Tropical species are typically chilling sensitive. At the plant level, chilling reduces germination, increases risk of seed attack by soil pathogens, reduced growth of root and shoot, stomata may remain open and abnormal flowers/fruits may be produced (they may even not be produced). Seed imbibitions and onset of photosynthesis in seedlings are the most sensitive to chilling. At the sub-cellular level, membrane damage is the primary effect of chilling; other effects include conformational change in proteins, reduced net photosynthesis, reduced chlorophyll synthesis, photosynthetic accumulation in chloroplasts, etc.

Chilling tolerance involves (i) membrane-lipid unsaturation (ii) reduce sensitivity of photosynthesis (iii) increase chlorophyll accumulation (iv) improved germination (v) fruit and seed set and, (vi) pollen fertility. Chilling tolerance is usually polygenic control and may be present in (i) well adopted breeding populations (ii) germplasm collections (iii) cold-tolerant mutants (iv) Soma-clonal variations and (v) related wild species. The selection may be carried out (i) under the field environment or (ii) in a controlled environment, e.g., in growth chamber.

Selection for chilling tolerance may be based on (i) germination (ii) growth under chilling stress (iii) chlorophyll loss due to chilling (iv) membrane stability (v) photosynthesis (vi) seeding mortality (vii) seed and/or fruit set and, (viii) pollen fertility.

Freezing stress is produced when plants are subjected to subzero temperatures. Dominant state is conducive to freezing resistance while resistance in actively growing tissues is rare. When water in plants cools below 0^0 C, it may form ice (requires a source of nucleation located outside e.g., dust particles, bacteria, etc., or inside plants), or super cool (up to -1 to -15^0 C) without ice crystal formation. Extra-cellular ice formation creates water stress in the frozen tissue. Initiation of ice formation on plant surface is sufficient to initiate freezing of internal water in most plant species.

Freezing resistance is a complex trait; it involves a variety of characters. It involves two distinct strategies, viz., (i) freezing avoidance (ability of tissues/organs to avoid ice formation of sub-zero temperatures, e.g., by super-cooling may be effective even up to - 47^0 C) and, (ii) freezing tolerance (results due to osmotic adjustment, bound water, plasma membrane stability, cell wall properties and cold responsive proteins). Freezing tolerance is preferable to freezing avoidance, particularly in regions where temperature may drop down to - 41^0 C.

Winter hardiness is in the order rye > wheat > barley > Oats. This trail is under polygenic control. The source for breeding tolerance may be (i) cultivated varieties (ii) germplasm collection (iii) mutants (iv) related wild species and, (v) transgenes (e.g., *ala* 3, a chemically synthesized antifreeze protein gene tested in tobacco). Selection for this trait may be done in the field

environment or in a controlled environment (these supplement the field evaluations). The selection criteria may be (i) field survival (ii) freezing test (iii) freezing of isolated crowns (in cereals) and (iv) osmo-regulation. According to one assessment little progress has been made in improving the winter hardiness beyond the level present in the varieties during the first half of the 20^{th} Century.

Mechanism of freezing resistance:

There is no single mechanism involved in plant freezing resistance or tolerance. But it does add to our understanding of how plants survive extremes of temperature.

Much plant damage in freezing temperatures is due to cell dehydration in which water is drawn out as it crystallizes and the organelle or cell membrane shrivels as liquid volume drops. Lipids in the membranes of tolerant plants are removed and converted to oil that accumulates in droplets retaining membrane integrity and conserving the energy by storing oil droplets. With rising global concern about water supplies and climate change, scientists see additional reasons to understand the ways hardy plants survive.

The advance researches lead to speculation that freezing itself can prompt cell proteins directly to change the composition of the membrane, without activation by gradual acclimation. This has been a major focus in the plant freezing resistance or tolerance field in last few years. The process of freezing resistance or tolerance through cold acclimatization is a two-stage mechanism.

1. The first stage occurs at relatively high subzero temperatures as the water present in plant tissues freezes outside the cell.

2. The second stage occurs at lower temperatures as inter-cellular ice continues to form within the apoplast. Antifreeze proteins localize the growth of ice crystals by ice nucleators in order to prevent physical damage to tissues and to promote super-cooling within freezing-sensitive tissues and cells. Osmotic stress including dehydration and high salinity, as well as treatment with abscisic acid, can also enhance freezing tolerance. Freezing tolerance can be assessed by performing a simple plant survival assay or with the more time consuming but quantitative electrolyte leakage assay.

Plants are not the only organisms capable of withstanding subzero temperatures. Animals like wood frogs, juvenile painted turtles, golden rod gall fly larvae and inter-tidal periwinkle snails have all been shown to be capable of the same. They convert up to 70% of their total body water into ice accumulating in extra-cellular spaces. In order to perform such remarkable acts, several biochemical adaptations have been identified as supporting factors to freeze tolerance. These include the following:

(a) Proteins: Nucleating proteins induce and regulate the whole process of extra-cellular freezing. Certain proteins, named ice restructuring proteins or antifreeze proteins stop small ice crystals from recrystallizing into larger crystals that can cause physical damage to tissues.

(b) Cryoprotectants: These are several factors that prevent intra-cellular freezing and also prevent excessive reduction of cell volume and, stabilize protein conformation. These cryo-

protectants commonly include high concentrations of polyhidric alcohols (glycerol, sorbitol, etc.) and sugars (glucose) that are packed into the cell. Other protectants are trehalose and proline which prevent the membrane bi-layer from collapsing.

(c) Ischemia tolerance: In order to survive cells and organs without circulation of the blood, good antioxidant defenses and elevated chaperone protein are required. Molecular chaperones are proteins that assists the conformational folding or unfolding of macromolecular structures. They help protect cell macro-molecules whilst metabolic rate depression greatly reduces cell energy needed whilst frozen.

Problems in breeding for freezing tolerance in plants:

Winter or freezing survival is a complex trait for breeders; the former is dependent on number of parameters including the morphological and physiological characteristics of the plant, soil conditions and weather fluctuations. Main problems associated with breeding for freezing tolerance in plants are as follows:

1. Germination and seedling establishment are sensitive growth stages for several plant species if we are using later on these plants for breeding to cold resistance or tolerance.
2. Cold and icy winds also contribute adversely during experimentation.
3. Cold temperature also adversely affects overall plant growth required for breeding experimentation.
4. Due to cold, freezing and low temperature male and female flower establishment is also poor which causes several problems for breeders like crossing, etc.
5. Effect of low temperature leads to poor microsporogenesis which led to severe male sterility ultimately hampering breeding results.
6. As we know temperature is optimum requirement for photosynthesis but during winter temperature is very low because of environment factors. This reduces growth and results in direct yield losses because there is less carbohydrate available for grain/seed production. Hence ultimately next year breeding evaluation trials are adversely affected due to lesser availability of seeds.

Heat Stress Resistance: The ability of some genotype to perform better than others when they are subjected to the same level of heat stress is known as heat stress resistance. The various mechanism of heat resistance may be grouped into two categories:

Heat avoidance: The ability of a genotype to dissipate the radiation energy and thereby to avoid a rise in plant temperature to a stress level is called **heat avoidance.** The primary mechanism of energy dissipation is **transpiration.** Transpiration cooling is the link between dehydration and heat avoidance so that under conditions of high solar radiation and water stress the effects of heat avoidance become inseparable from those of dehydration avoidance.

Heat Tolerance: Ability of some genotypes to withstand or perform better than others when their internal temperatures are comparable and in the realm of heat stress is called heat tolerance. Heat tolerance is largely associated with the cellular and sub-cellular components and, its expression is

highly dependent on heat hardening or heat acclimatization. Heat hardening may be defined as an improved ability of a genotype to withstand a period of high temperature as a consequence of an earlier exposure to high temperatures for a given period of time. Heat tolerance may involve the following components: (i) membrane stability (ii) reduced heat sensitivity of photo system II (iii) photosynthate translocation (iv) stem reserve mobilization and, (v) osmo-regulation.

TABLE: 1 Different heat stress mechanism in plants

S. No.	Mechanism	Contributory processes	Consequences/remarks
1.	Heat avoidance	Transpiration Leaf reflectance due to: 1. Pubescence 2. Glaucousness 3. Insulation by bark	 Reduces light interception by leaves Reduces heating
2.	Heat Tolerance	Membrane stability Stability of photo system II Photosynthate translocation Stem-reserve mobilization Osmo-regulation	 Associated with heat hardening and possibly Heat Shock Protein synthesis

Breeding strategy for Temperature Stress:

Understanding the effects of temperature on phenology and the physiological traits linked to tolerance is imperative if plant breeders are to develop cultivars better adapted to these hostile conditions. For example, while it is well-known that crop flowering and maturity are accelerated by high temperature, heat stress also affects photosynthesis, membrane stability, pollen fertility, fruit and/or seed yield, depending on plant species and stress intensity. Furthermore, recent advances in molecular technology have broadened the breeding strategies available to improve heat tolerance. Several crop genomes have been sequenced and a number of others are in progress, thus tools for comparing genomes and evaluating transcriptome response to abiotic stress are available. Gene discovery methods and transgenic plants have helped to understand physiological traits involved with stress tolerance and to move tolerance genes between species. In addition, heat-stress tolerance is multigenic and can now be manipulated rather than only one gene at a time. These methods show potential for modifying and combining genes to meet the heat-tolerant crop needs of the future

Breeding methods and approaches used for drought resistance or tolerance:

Breeding for drought resistance or tolerance refers to breeding for yield under soil moisture stress conditions. It also refers to yield improvement in environment represented by water deficit. Basically four breeding methods are used:

1. Introduction
2. Selection

3. Hybridization
4. Mutation

In self pollinated crops like paddy, wheat, groundnut and jute, introduction, pure line selection, mass selection, hybridization (pedigree method and back- cross) and, mutation breeding are used. Simple screening is effective in selection of drought tolerant genotype in wheat under mild drought conditions. Careful selection of parents, combining several drought resistant characters in one genotype, screening of material under drought conditions, use of large population in yield trials and testing at several locations will help in selection of superior lines.

In cross pollinated crops like maize and sugarcane etc, the most commonly used methods are mass selection, back cross hybridization of inbreds to develop hybrid cultivars, recurrent selection and formation of synthetic cultivars.

6.4 RESISTANCE BREEDING FOR FROST

The term “frost tolerant” means that a plant has a high hardiness rating and can withstand not only freezing temperatures but will also likely to survive light to moderate frosts which can kill plants that are cold tolerant, but not frost tolerant. Freeze damage to plant tissue can be detrimental to plants. Light frost typically doesn't cause major damage with exception to very tender plants but hard frost freezes water in plant cells, causing dehydration and damage to cell walls.

In temperate climate, winter crops are the majority and frost damage is an important factor reducing crop yields, especially in regions where winter is regularly severe. The effects of winter climate change on crops is however under represented compared to studies on crop-related climate impact research during spring and summer seasons; in particular the paradoxical increase in spring freezing injury (observed/simulated) with warming for cold climate vegetation should be documented for winter crops.

The response of crops to winter temperature under a warming climate is indeed complex and varied. The acclimation process allows a plant to get more resistant to frost. It requires sufficient low temperature during sufficient time. The opposite process *de-acclimation* occurs when temperature rises. The main plant characteristics determining the frost resistance of winter crops are the variety, being more or less resistant to frost and having different acclimation rates and the plant stage. The main environmental factor determining the frost response is the temperature. These eco-physiological processes have been synthesized in a predicting crop model for frost resistance.

Bed sheets, drop cloths, blankets and plastic sheets make suitable covers for vulnerable plants. The coverings should be removed when temperatures rise the next day. For a short cold period, low plantings can be covered with mulch, such as straw or leaf mold.

The various components of frost tolerance are as follows:

1- Osmotic Adjustment: In many cereal plants, fructans become converted to fructose and sucrose during frost stress and the ratio of this conversion is considered as an index of frost

tolerance in such crops. In addition, accumulation of solutes like proline and trehalose also offers protection to cell membranes during frost attack.

2- Bound water: A part of cell water is bound in such a way that it does not participate in osmo-metric response: such water may form up to 1/3 of total cellular water. Bound water increase frost tolerance.

3- Plasma Membrane Stability: During frost-hardening, plasma membrane fluidity increases and its sensitivity to frost induced injury decreases. The increased stability of plasma membrane prevents the extension of ice formation to intra-cellular water; this allows future super-cooling of the cellular solution and reduces the frost temperature of the cells. Plasma membrane injury be freezing stress can be measured by the rate of electrolyte leakage.

4- Cell Wall Properties: When inter-cellular space is limited then during frost attack, water freezing in the cell wall is affected by the size of cell wall micro capillaries. Xylem mucilage of the cell wall inhibit the kinetics of frost attack process, this effect depends mainly on the type of mucilage present in cell walls e.g., rye mucilage is more effective than barley mucilage.

5- Cold-Responsive Proteins: A number of proteins are produced in response to low temperature due to frost attack. These proteins are called cold-responsive proteins. The precise role of these proteins in metabolism and cold tolerance is yet to be elucidated but these may well enhance frost tolerance.

Breeding Strategies for Frost Stress:

It is being observed that the possible range of osmotic adjustment is most appropriate method for frost stress tolerance. The genetic resources required in breeding for frost stress may be found in (1) cultivated varieties (2) germplasm (3) mutagensis (4) related wild species and (5) other organism (transgenic breeding).

Advancement in plant biotechnology, molecular markers and genomics have enabled breeders to formulate new tools for the analysis and manipulation of genetic variability and the development of improved plant types for frost stress. Molecular tools are being increasingly used in plant breeding to widen its impact for meeting the global needs for sustainable increases in agricultural productivity by making plants tolerant to frost stress.

Problems in Breeding for Frost stress tolerance:

(i) Frost tolerance is a complex trait and it involves several components. Therefore, it cannot be readily measured especially under the field environments.

(ii) Field survival shows poor heritability and breeding efforts are often frustrating.

(iii) Frost stress tolerance also shows a large G x E interaction which limits progress under selection.

6.5 SALT AND ACID TOLERANCE

Salinity is a major abiotic stress limiting growth and productivity of plants in many areas of the world due to increasing use of poor quality of water for irrigation and soil salinity. Plant

adaptation or tolerance to salinity stress involves complex physiological traits, metabolic pathways, and molecular or gene networks. A comprehensive understanding on how plants respond to salinity stress at different levels and an integrated approach of combining molecular tools with physiological and biochemical techniques are imperative for the development of salt-tolerant varieties of plants in salt-affected areas. Salt tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt. Plants that can survive on high concentrations of salt in the rhizosphere and grow well are called halophytes.
Salinity resistance may arise from:

1. Resistance to water stress or osmotic stress or osmotic effects of salinity.
2. Resistance to salinity-induced ionic toxicity or toxic effects of salinity or a combination of both. Genetic variation for salinity resistance exists both among and within species. A reliable estimation of the magnitude of this variability, especially the within species variability, is critical to the success of breeding program for salinity resistance. Information on inheritance of salinity resistance is rather limited most likely due to the complex nature of salinity resistance and the difficulties in creating precisely controlled and dependable salinity environments.

The various approaches for the development of salinity tolerant cultivar may be listed as follow: (i) Use of tolerant root stocks (ii) Selection (iii) Hybridization (iv Inter specific hybridization (v) Cell selection and, (vi) Genetic engineering.
There are varietal differences in plant tolerance against severe acidity in some crop species like oats, triticale, rye and wheat. Almost all oats, triticale, narrow leaf lupin and cowpea varieties for example, have excellent tolerance to acid top and sub-soils. Plant nutrients leach from the soil much faster at pH values below 5.5 than from soils within the 5.5 to 7.0 range. In some mineral soils aluminum can be dissolved at pH levels below 5.0 getting toxic to plant growth. Soil pH may also affect the availability of plant nutrients. Different factors responsible and associated with soil acidity are considered to be limiting for plants in many parts of world. Research on acid-tolerance mechanisms of soil grown plants will have important implication in facilitating soil and crop management in the tropical and subtropical regions. Soil becomes acidic due to natural soil acidification process. In acidic soils, toxicity is common due to solubilisation of Aluminium $^{3+}$ ion which is toxic for plants. The main symptom of Al^{3+} toxicity is the inhibition of root growth. After Al^{3+} toxicity, Manganese toxicity ranks as the second major concern for plant growth in acid soils. Unlike Aluminum, Manganese is an essential plant nutrient and is toxic when taken up in excessive quantities by plants. In addition to Al and Mn toxicity, low pH also affects plant growth in highly acid soils. Low pH stress facilitates H+ influx into root tissues resulting in poor plant growth. However, phosphorus (P) deficiency is also common in acid soils. Phosphorus leads to an Al-phosphate complex in acid soils that result in less P availability in these soils. Different Al tolerance mechanisms such as an increase in rhizosphere pH, plant tissue tolerance, exudation of organic acid and role of apoplast is very important in this aspect. So it can be concluded that P may play an important role in Al tolerance. Thus, understanding acid tolerance mechanisms in soil

grown plants has important implications for sustainable agriculture in the tropical and subtropical regions.

Breeding Strategies for Salt and Acid Tolerance:

The different strategies used for the development of salinity and acid resistant /tolerant varieties of different plants are as follows:

(1) Use of resistant root stock
(2) Selection
(3) Hybridization
(4) Inter-specific hybridization
(5) Cell selection
(6) Genetic engineering

(1) **Use of Salinity and acid tolerant root stock:** This approach is applicable for horticultural crops. For example salinity resistant root stocks have been used for grapes and this is considered as a significant contribution to grape industry.

(2) **Selection:** Selection has been quite effective in development of salinity and acid resistant lines with improved performance under both stress conditions.

(3) **Hybridization:** Intervarietal hybridization is used to combine salinity and acidity resistance present in one parent with high yielding ability of the other parent. The segregating generations are generally handled according to the pedigree method.

(4) **Inter-specific Hybridization:** Interspecific hybridization resistance technique has been attempted in different crops like potato, tomato, brinjal, wheat, etc., for instance in tomato variety, "Walter" was crossed with salinity resistant species *Lycopersicon cheesmanii* ssp. *minor*. F_2 and subsequent generations were subjected to salinity tress by germinating the seeds in the presence of NaCl (up to 300 mM). The surviving seedlings were saved by eliminating NaCl in a stepwise fashion and grown to maturity in the field.

Salinity Tolerance Mechanism in Plants:

Plant develop various physiological and biochemical mechanisms in order to survive in soils with high salt concentrations. Principle mechanisms include, but are not limited to homeostasis and compartmentalization, ion transport and uptake, biosynthesis of osmoprotectants and compatible solutes, activation of antioxidant enzyme and synthesis of antioxidant compounds, synthesis of polyamines, generation of nitric oxide (NO) and hormone modulation.

6.6 LODGING

Lodging in cereal crops is a major problem that results in decreased grain yield and deteriorated grain quality. The primary causes of plant lodging include legion, increased nitrogen levels, over plant population, soil density, diseases, natural disasters such as storm damage, sowing date and seed type, are all mainly contributing factors to lodging in cereal crops. Lodging refers to stem breaking type and stem bending type (stem lodging) or root lodging (anchorage failure) of the

plants, and is one of the most concerning problems faced by the farmers worldwide. Generally, the possibility of lodging occurs when the plant weight (upper parts of plants) increased by the interception of rainfall. When the lower stem parts are weakened by disease attack or by overdose application of nitrogenous fertilizer, or when the shearing cohesive bond strength of the soil particles around the root system is almost completely deteriorated by rainfall. Lodging severely affects grain production of the major cereal crops. Wheat and rice for example, also have several other indirect knock-on effects such as crop harvest at a slower pace, reduction in grain quality and drying costs. These factors are considered among some of the major constraints in reducing crop productivity globally.

Development of lodging-resistant varieties to cope this challenge has been widely attended to increase yield in maize, rice and other crops. Plant breeders have mitigated lodging risk by introducing the semi-dwarf gene *sd1*, known as the "*Green Revolution Gene*". However, recent studies showed that semi-dwarf trait in rice limits photosynthesis and biomass production leading to an yield penalty. Additionally, it may have a negative pleiotropic effect on culm morphology. In other words, the gibberellin synthesis gene (*sd1*) widely applied in Green Revolution rice also reduces the culm strength by decreasing culm diameter which makes it difficult to further improve lodging resistance by one semi-dwarf genes alone. Thus, it is important to search for alien genes favourable for breeding lodging-resistant rice.

Lodging resistance is a complex quantitative trait, which is affected by many factors, such as culm morphology, culm diameter and length, cellulose content, and environment conditions. Previous studies have shown that the culm diameter and size are highly correlated with the lodging resistance of rice. Figure-1 describes different approaches for developing plants for lodging resistance.

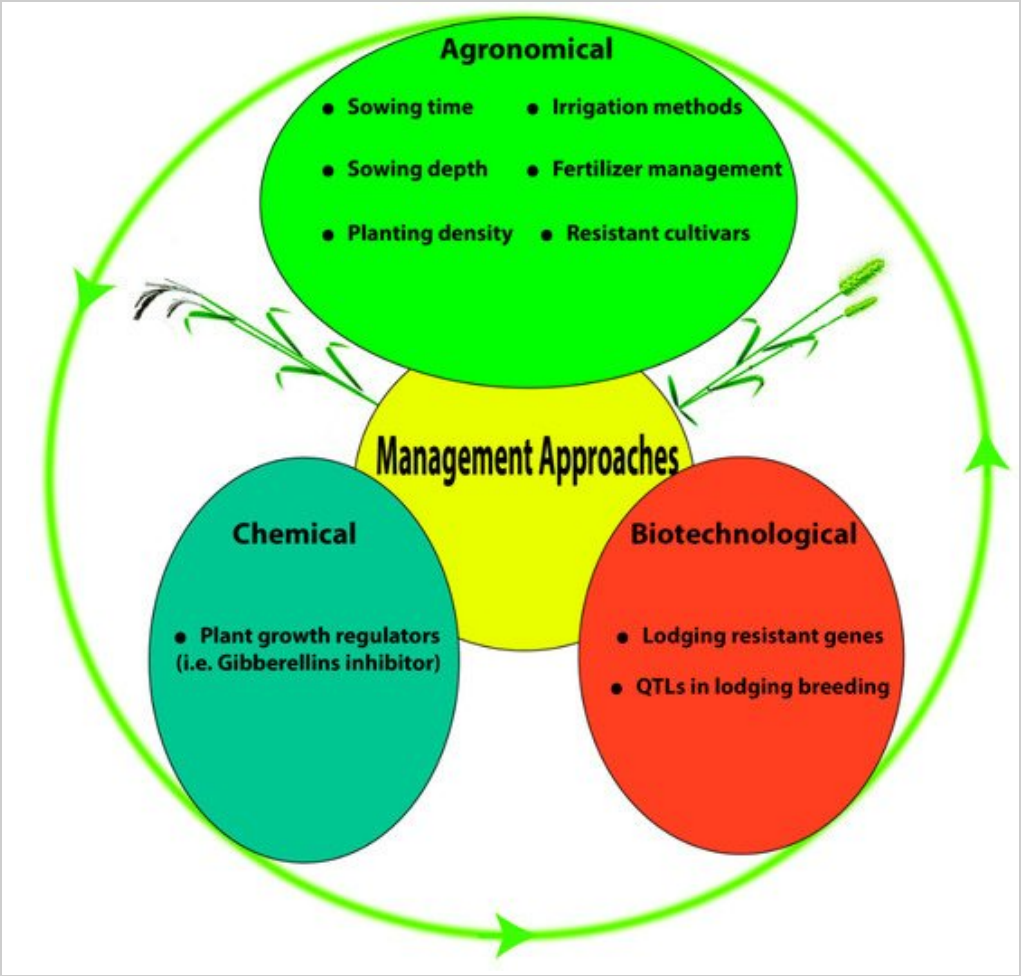

Fig.6.1: Collective approaches for developing lodging resistant plants by molecular breeding strategies, chemical control and agronomical management approaches applying to mitigate field lodging.

Breeding Strategies for Lodging Resistance:

Scientists studied lodging resistance related traits for establishing breeding program. Negative correlation between inter-node length and lodging resistance is being observed in different crop plants like rice and wheat. Lodging resistance related traits like culm thickness, culm strength, basal inter-nodal length and per cent of lodging expressed duplicate type of epistasis.

Parental lines and hybrid combinations with culm characteristics of middle plant height, roundish stem, shorter basal inter-node and longer top 1st inter-node should be selected to improve lodging resistance in super high yield hybrid rice breeding.

Pedigree analysis showed lodging resistance of varieties is closely correlated with that of their parents. Among the parental traits, plant height, inter-node lengths, thickness of inter-node wall and diameter of node have significant correlation with lodging resistance.

Analysis of Quantitative Trait Loci (QTLs) can reveal genetic basis of relationships among traits and allow comprehensive investigation of the genetic relationships among the morphological and physiological traits.

In cereal crops, considerable loss in yield and quality occurs due to lodging at reproductive phase. So far breeders have countered lodging risk by developing dwarf plants. Despite short stature, plant varieties of cereals like wheat and rice prone to lodging under intensive agricultural management practices. Therefore plant breeders must exploit wide genetic variation for strong culm traits for the development of non-lodging varieties.

6.7 RESISTANCE BREEDING FOR DISEASES

Plant breeders emphasize selection and development of disease resistant plant lines. Plant diseases can also be partially controlled by use of pesticides and by cultivation practices such as crop rotation, tillage, planting density, disease free seeds and cleaning of equipment. Plant varieties with inherent (genetically determined) disease resistance are generally preferred. Breeding for disease resistance began when plants were first domesticated. Breeding efforts continue because pathogen populations are under selection pressure for increased virulence, new pathogens appear, evolving cultivation practices and changing climate can reduce resistance and /or strengthen pathogens and plant breeding for other traits can disrupt prior resistance. A plant line with acceptable resistance against one pathogen may lack resistance against others.

Breeding for resistance typically includes:

- Identification of plants that may be less desirable in other ways, but which carry a useful disease resistance trait including wild plant lines that often express enhanced resistance.
- Crossing of a desirable but disease-susceptible variety to a plant that is a source of resistance.
- Growth of breeding candidates in a disease-conducive setting, possibly including pathogen inoculation. Attention must be paid to the specific pathogen isolates, to address variability within a single pathogen species.
- Selection of disease- resistant individuals that retain other desirable traits such as yield, quality and other disease resistance traits.

Resistance is termed durable if it continues to be effective over multiple years of widespread use as pathogen populations evolve. "Vertical resistance" is specific to certain races or strains of a pathogen species and is often controlled by single R genes and, can be less durable. Horizontal or broad spectrum resistance against an entire pathogen species is often only incompletely effective but more durable and, is often controlled by many genes that segregate in breeding populations.

Crops such as apple, banana, potato and sugarcane are often propagated by vegetative reproduction to preserve highly desirable plant varieties, because for these species, out-crossing seriously disrupts the preferred traits. Vegetative propagating crops may be among the best targets

for resistance improvement by the biotechnology method of plant transformation to manage genes that affect disease resistance.

Scientific breeding for disease resistance originated with Sir Rowland Biffen, who identified a single recessive gene for resistance to wheat yellow rust. Nearly every crop was then bred to include disease resistance (R) genes, many by introgression from compatible wild relatives.

There are three different modes of inheritance.

I. **Oligogenic Resistance:** When the resistance is governed by one or few major gens, it is known as oligogenic resistance. In such cases, each gene has large and easily identifiable effect on resistance. The resistance may be governed by dominant or recessive genes.

II. **Polygenic Resistance:** In some cases, the disease resistance is governed by several genes each having small and generally additive effect. The effect of each gene is not easily detectable. This type of resistance is referred to as polygenic resistance or quantitative resistance.

III. **Cytoplasmic Resistance:** Sometimes, disease resistance is governed by cytoplasmic genes.

There are two types of genetic resistance depending upon the number of races controlled.

(a) Vertical or specific Resistance: Specific resistance of a host to the particular race of a pathogen is known as vertical resistance. This type of resistance is governed by one or few genes and therefore is referred to as oligogenic resistance. When the resistance is controlled by single gene, it is called monogenic resistance. Since vertical resistance controls only one race of a pathogen it is also termed as specific resistance. Because of its simple inheritance, it is known as qualitative resistance. The host with vertical resistance controls only one race; therefore, it is also known as non uniform resistance.

(b) Horizontal or general resistance: The resistance of a host to all the races of a pathogen is called horizontal resistance. This type of resistance is called by various names as per reasons given below:

1. ***General Resistance*:** The host plant provides protection from all the prevailing races of a pathogen.

2. ***Polygenic Resistance*:** The resistance is controlled by a number of genes and also called quantitative resistance.

3. ***Minor Gene Resistance*:** Each gene involved in the resistance has small effect which is not visible

4. ***Non-specific resistance*:** The host resistance is not for a specific race of a pathogen.

The resistance is similar to all the races of a pathogen, hence also called as uniform resistance.

GENE-FOR-GENE-HYPOTHESIS:

The concept of gene for gene hypothesis was first developed by Flor in 1956 based on his studies of host pathogen interaction in flax for rust caused by *Malampsora lini*. The gene for gene hypothesis states that for each gene controlling resistance in the host, there is a corresponding gene controlling pathogenicity in the pathogen. The resistance of host is governed by dominant genes and virulence of pathogen by recessive genes. The genotype of host and pathogen determine the disease reaction. If some gene loci remain unmatched, the host will show resistance reaction (Table-2). The gene for gene hypothesis is also known as "Flor Hypothesis".

TABLE 2. A simple way to explain gene for gene relationship hypothesis.

Varieties	Host genotype	Pathogen genotypes	Disease reaction
1.	AA	Aa	Susceptible
	Aa		
	BB	bb	Susceptible
	Bb		
	CC	cc	Susceptible
	Cc		
2.	AA BB	Aa or bb	Resistant
	AaBB		
	AaBb	aabb	Susceptible
	AACC	aa	Resistant
	AaCC	cc	Resistant
	AaCc	aacc	Susceptible
3.	AA BB CC	aa bb	Resistant
	AABBCC	aa cc	Resistant
	Aa Bb Cc	aabbcc	Susceptible

Breeding Strategies for Disease Resistance:

Breeding methods for disease resistance are the same as for other agronomic characters. Main breeding methods are as follows.

1. **Introduction:** This is easy and rapid method of developing disease resistant variety. A resistant variety may be introduced and after testing, if found suitable, can be released in disease prone area.
2. **Selection:** When the source of resistance is a cultivated variety, mass selection and pure line selection in self pollinated crops; mass selection and recurrent selection in cross pollinated

species and, clonal selection in the vegetatively propagated crops will be ideal for isolating disease resistant plants.

3. **Hybridization:** Hybridization is used when resistant genes are available either in the gremplasm or in wild species of crop plants. After hybridization, the hybrid material is handled either by pedigree method or by back cross method. The pedigree method is used when the resistance is governed by polygenes and the resistant variety is an adopted one which also contributes some desirable agronomic traits. The back cross method is used when the resistant parent is unadopted type or the resistant gene is to be transferred from wild species.

6.8 SUMMARY

Breeding for drought resistance is an important objective of plant breeding program in many crops. If we take example of our country as in India about 73.5% of the agriculture is represented by dry land farming. In rainfed areas, crops generally suffer from drought at one or the other stage. Hence, breeding work on drought resistance is carried out in almost all the National and International Crop Research Institutes. In India, drought and salinity resistant or tolerant varieties have been developed in several crops.

In India, breeding work for salinity resistance is mainly carried out at CSSRI (Central Soil Salinity Research Institute), Karnal (Haryana). Salinity resistant varieties have been developed in barley, okra, onion, rice and sugarcane crops in India.

Disease resistant varieties have been developed in many crops all over the world. In India, disease resistant varieties have been evolved in barley, cotton, maize, oilseeds, pulses, rice, sorghum, sugarcane, wheat, and many other crops. Almost all the currently released varieties of *arboreum* cotton are resistant to *Fusarium* wilt. Many varieties of wheat are resistant to rusts. In sugarcane, several varieties are resistant to red rot and wilt. In okra, a yellow mosaic virus resistant variety Prabhani Kranti has been released .In upland cotton variety MCU 5 VT is tolerant to *Verticillium* wilt.

6.9 GLOSSARY

Abiotic stress: Adverse conditions for crop growth and production caused by environmental factors such as deficiency or excess of nutrition, moisture, temperature and light; the presence of harmful gases or toxicants, and abnormal soil conditions such as salinity, alkalinity and acidity.

Antibiosis: Adverse effects of the host on feeding, development and reproduction of insect pests

Biotic stress: Adverse conditions for crop growth and production caused by biological factors such as diseases, insects and parasitic weeds.

Disease: In crop plants various disorders caused by pathogens are called disease.

Drought: Condition of soil moisture deficiency or water scarcity.

Drought avoidance: Ability of plants to maintain a favorable internal water balance under moisture stress.

Drought hardening: Improvement in drought tolerance ability of a genotype through various seed and seedling treatments.

Drought resistance: Ability of crop plants to grow, develop and reproduce normally under moisture deficit condition or survival of plants under water deficit conditions without injury. It is a sum of drought avoidance and drought tolerance.

Drought tolerance: Ability of plants to withstand low tissue water content.

Epidemic: Wide spread uncontrolled incidence of a disease in known as epidemic.

Gene for gene hypothesis: This hypothesis states that for each gene controlling resistance in the host, there is a corresponding gene controlling pathogenicity in the pathogen: also called Flor Hypothesis after the name of Scientist who developed this concept.

Gene deployment: Planned geographical distribution of major genes for specific resistance to pests for use in varietal development and production

Gene pyramiding: Incorporation of two or more major genes in a variety for specific resistance to a pest.

Genetic resistance: Ability of some genotypes to give higher yield of good quality than other varieties at the same initial level of diereses or insect infestation under similar environmental conditions. It is of two types, *viz.* vertical and horizontal.

Horizontal resistance: Resistance of a host to all the prevalent races of a pathogen; also called minor gene resistance, polygenic resistance, non specific resistance, general resistance

Host: The plant which is attacked by a disease, insect or parasitic weed is called host.

Hydroponics: Growing of plants in nutrient solution.

Hypersensitivity: A host pathogen reaction which leads to death of infested tissues.

Immune: Completely or fully resistant plants are known as immune one.

Lysimeter: Cemented micro-plots of various sizes used for study of roots and salt tolerance

Parasite: An organism or virus which lives upon or within another living organism is called parasite

Pest: Any animal or higher plant which parasitizes crop plants *i.e.* insects, nematodes, and parasitic weeds refer to pest.

Pathogen: Various disease causing organisms such as bacteria, fungi, mycoplasma and viruses refer to pathogen

Pathogenecity: Ability of a pathogen to attack a host is called pathogenecity

Porometer: An instrument that is used for measuring stomatal aperture.

Salt tolerance: Ability of plants to prevent, reduce or overcome the injuries effects of soluble salts present in the root zone

Stress: Advance conditions for crop growth and production imposed either by biotic or abiotic factors

Tolerance: Ability of a host to reproduce well despite the establishment of a pathogen in the host tissues or the ability of a variety to produce more yield than susceptible variety at the same level of disease attack

Vertical resistance: Resistance of a host to the particular race of a pathogen. Also called major gene resistance, oligogenic resistance, and qualitative resistance

Virulent: A race of a pathogen capable of attacking a host with specific resistance.

6.10 SELF- ASSESSMENT QUESTIONS

6.10.1 Fill in the blank:

1. Condition of soil moisture deficit is referred to as……
2. Ability of plants to maintain a favorable internal water balance under moisture stress is known as……..
3. Ability of plants to withstand low tissue water content is called……
4. Ability of plants to grow, develop and reproduce under moisture deficit conditions is termed ……..
5. Long lasting resistance is referred to as……..
6. Resistance exhibited by young seedlings is called ……..
7. Resistance exhibited by adult plants is referred to as …….
8. ….…….. is a race of a pathogen which is capable of attacking a host with specific resistance
9. Condition of soil moisture deficiency or water scarcity is known as……..
10. Adverse conditions for crop growth and production imposed either by biotic factors is called……..
11. Ability of some genotypes to give higher yield of good quality than other varieties at the same initial level of disease attack under similar environmental conditions is known as……..
12. Resistance of a host against a particular race of pathogen is called……
13. ….……..resistance refers to resistance of a host to all the prevalent races of a pathogen.
14. The terms vertical and horizontal resistance were coined by……. in……..
15. Various disease causing organisms, such as bacteria, fungi, mycoplasma and viruses are known as…….
16. The concept of gene for gene hypothesis was developed by…….in…….
17. The gene for gene hypothesis is also known as……..

Answer Key 6.10.1: 1-Drought, **2-** Drought avoidance, 3- Drought tolerance, 4- Drought resistance, 5- Durable resistance, 6- Seedling resistance, 7-Adults resistance, 8-Virulent, 9-Drought, 10-Biotic stress, 11-Genetic resistance, 12-Vertical resistance, 13- Horizontal resistance, 14-Van der Plank , 1963, 15- Pathogens, 16-Flor (1956), 17-Flor Hypothesis

6.10.2 Multiple Choice Questions:

1- The term vertical resistance and horizontal resistance were coined by

(a) Robinson (1971) (b) Vander Plank (1963)

(c) Russell (1978) (d) Nelson (1973)

2- Vertical resistance is also known as
(a) Major gene resistance (b) Race specific resistance
(c) Oligogenic resistance (d) All of the above

3-Horizontal resistance is also referred to as
(a) Minor gene resistance (b) General resistance
(c) Polygenic resistance (d) All of the above

4- Gene for Gene hypothesis was proposed by
(a) Nelson (1973) (b) Flor (1956)
(c) Van der Plank (1963) (d) Robinson (1971)

5-Concept of gene for gene hypothesis was developed in
(a) Pearl Millet (b) Sorghum
(c) Linseed (d) Garden Pea

6-In wheat, stem sawfly has non-preference for
(a) Small leaves (b) Thick leaves
(c) Solid stem (d) All of the above

7-Ability of plants to maintain a favorable internal water balance under moisture stress is known as
(a) Drought escape (b) Drought tolerance
(c) Drought avoidance (d) Drought hardening

8- Lysimeter is used to measure
(a) Stomatal aperture (b) Tissue water potential
(c) Rate of photosynthesis (d) Salt tolerance

9-Psychrometer is used to measure
(a) Rate of photosynthesis (b) Salt tolerance
(c) Stomatal aperture (d) Tissue water potential

10-Pitic is a drought resistance variety of
(a) Barley (b) Wheat
(c) Maize (d) Sorghum

11-Mohan variety of rice is tolerant to
(a) Drought (b) Salinity
(c) Metal toxicity (d) All of the above

12-Under drought conditions, there is an increase in
(a) Abscisic acid content (b) Ethylene level

(c) Proline level (d) All of the above

Answer Key 6.10.2: 1-(b), 2-(d), 3-(d), 4- (b), 5-(c), 6-(c), 7-(c), 8-(d), 9-(d), 10-(b), 11-(b), 12-(d)

6.10.3 Very Short Answer Type Questions:

a) Define antibiosis.
b) What is basic difference between vertical resistance and horizontal resistance?
c) What do you know about hypersensitivity?
d) What is tolerance?
e) Define gene for gene hypothesis?
f) Which instrument is used to measure tissue water potential in plants?
g) What is drought avoidance?
h) What is epidemic?
i) What is gene deployment?
j) What is gene pyramiding?
k) What does frost tolerant mean?
l) How frost hurts plants?
m) How can you protect your plants from frost?
n) How do salt tolerant plants work?
o) What is the mechanism of the salt tolerance?
p) What are acid tolerant crops?

6.10.4 Short Answer Type Questions:

1. What is stress?
2. What are biotic factors?
3. What are abiotic factors?
4. What is the mechanism of drought resistance?
5. What is drought avoidance?
6. What is drought tolerance?
7. What is drought resistance?
8. What is moisture stress?
9. What is the main difference between drought avoidance and drought tolerance?
10. What is genetic resistance?
11. What are basic difference between vertical resistance and horizontal resistance?
12. What is salt tolerance in plants?
13. What are acid tolerant crops?
14. How does acidic soil affect plant growth?
15. What is different mechanism of heat stress in plants?
16. What are different breeding strategies for salt and acid tolerance?
17. Explain with help of diagram, different strategies for developing plants for lodging resistance.

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6.13 TERMINAL QUESTIONS

1- Describe different types of resistant breeding methods in plants.

2- What are basic differences between different types of gene resistance mechanisms?

3- Illustrate mechanism of disease resistance in plants.
4- Write elaborately comparative analysis of drought tolerant measures.
5- Describe the applications of in resistance breeding in Crop Improvement.
6- Differentiate between resistance and tolerance.
7- Differentiate between drought avoidance and drought tolerance
8- What do you know about genetic basis of resistance?
9- What are various components of frost tolerance?
10- Define temperature stress and give its various types. Explain the meaning and the consequences of heat stress.
11-What are different breeding strategies for developing resistance against diseases?

BLOCK-3-CYTOGENETICS

UNIT-7 DEVELOPMENT OF GENETICS

7.1 *OBJECTIVES*

After reading this unit student will be able to:

- Explain and define the meaning of genetics.
- Describe the gradual development of genetics in chronological order.
- Explain the pre-mendelian theories and post-mendelian developments in genetics.
- Describe the molecular era of genetics

7.2 *INTRODUCTION*

In the previous blocks you have studied the characteristic features of plant breeding, its concept, objectives, methods, achievements and scope. You also learnt about male sterile, heterosis breeding and apomixes. In the previous unit you studied about the resistance breeding of plants for tolerance to environmental factors and diseases. The aim of plant breeding is to make genetic manipulations in plants to achieve targeted and directional changes in the nature of plants to make them more useful to humans. For this purpose you should have knowledge about genetics. In this link, there is next branch of biological science called genetics which is the study of heredity and variation. The term 'Genetics' came from the Greek word 'gen' which means to generate or to grow or to become something. This term was used for the first time by W. Bateson in 1905.

Heredity includes those traits or characters which are transmitted from generation to generation. Heredity means that all living things tend to produce young ones like themselves. For example, cat can produce only a cat; a horse can produce a little horse but never a tiger. Similarly apple, tomato and grape plants can produce only apple, tomato and grapes respectively. The progeny may be similar to their parents but they are never exact repetitions. They differ not only from one another but from their parents also in many characters. These differences are called **variations** which may be of two types: (i). **Hereditary Variations** which are result of sexual reproduction and inherited from one generation to other, and; (ii) **Environmental Variations** which are non-heritable and appear in the organisms when they grow in the changed environmental conditions, such as temperature, food, moisture, light and so on.

Gregor John Mendel is known as the **Father of Genetics**. He was an Austrian monk who formulated the laws of heredity after conducting simple hybridisation experiments on pea plants (*Pisum sativum*) in the garden of his monastery. His work is the basis of understanding the principles of genetics.

7.3 MENDELIAN INHERITANCE

Mendel's experiments on the garden pea are regarded as a great landmark in the study of genetics. Mendelian inheritance, also called **Mendelism**, is the principles of heredity

formulated by Gregor John Mendel in 1865. He chose common garden pea plants for his research because they can be grown easily in large numbers and their reproduction can be manipulated. Pea plants have both male and female reproductive organs. Therefore, they can either self-pollinate or cross-pollinate with another plant. He considered seven pairs of contrasting characters in his experiments that are easily recognised- length of the plant, either tall or dwarf; position of the flowers, either axial or terminal; color of the flower, either purple or white; colour of the unripe pods, either yellow or green; shape of unripe pods, either inflated or constricted; shape of the seed, either rounded or wrinkled; and colour of the seed, either yellow or green.

On the basis of results obtained from his experiments, Mendel gave four postulates. He believed that an equal contribution of the two parents was necessary for the development of characters in hybrid generation. His postulates are as follows:

1-Principle of Unit Factors- Mendel gave the idea of hereditary units, which he called "**factors**" which are responsible for growth of various characters. These factors, now called as genes, are transmitted from one generation to the next. These factors occur in alternative forms and are responsible for variations in inherited characters. For example, the gene for flower color in pea plants exists in two forms, one for purple (PP) and the other for white (pp). The alternative "forms" are now called alleles. When an organism has two same alleles (PP or pp) for a gene, then it is called **homozygous** for that gene. If an organism has two different alleles (Pp) for a gene, then it is said to be **heterozygous** for that gene.

2- Principle of Dominance- On crossing two parent plants, character of only one of the two parents is expressed in the first generation (F_1). This is known as **dominant** character. Character of the other parent is not expressed in F_1, such a character is known as recessive character. Mendel in his experiment clearly established the fact that the recessive characters are not expressed in heterozygous condition, but will be expressed in recessive homozygotes only. For example, in cross-pollinating plants that either produces yellow or green pea seeds, the first offspring generation (F_1) always has yellow seeds. It means that yellow seed color is dominant. However, in the following generation (F_2), a 3:1 ratio of yellow to green was consistently found. Mendel made a clear distinction between the appearance or observable physical characters (Phenotype) and the actual genetic makeup (Genotype) of plants.

3-Law of Segregation or Law of Purity of Gametes- This is **Mendel's First Law of Inheritance** which states that the alleles of a gene separate or segregate from each other during the formation of gametes. Therefore, a male or female gamete carries only one allele for each inherited trait. For example- pea plant with purple flowers has two alleles 'PP'. During gamete formation these alleles will separate i.e. one 'P' will go to female gamete and the other 'P' will go to the male gamete.

4- Law of Independent Assortment or Mendel's Second Law of Inheritance states that alleles for separate traits are passed independently of one another from parents to offspring. It means that if a cross is performed between two plants differing in two characters, also known as a dihybrid cross, then the alleles for one character will be separated

independently of the alleles of the second character during gamete formation. For example, Mendel chose to cross a pea plant that was homozygous and dominant for round (RR), yellow (YY) seeds with a pea plant that was homozygous and recessive for wrinkled (rr), green (yy) seeds. The offspring of the RRYY x rryy cross, which is called the F_1 generation, were all heterozygous plants with round, yellow seeds and the genotype RrYy. A ratio of 9:3:3:1 was obtained in F_2 generation of his dihybrid cross and produced nine plants with round, yellow seeds, three plants with round, green seeds, three plants with wrinkled, yellow seeds and one plant with wrinkled, green seeds. From his experiment, Mendel concluded that the pairs of traits in the parental generation inherited independently from one another, from one generation to the next.

These experiments led Mendel to formulate the fundamental laws of inheritance. He presented the facts and figures of his experiments before the Brunn Natural History Society on February 8, 1865. His 46-paged paper, entitled "Experiments in Plant Hybridization", was published in the Proceedings of the Brunn Society for the Study of Natural Sciences in the following year, in 1866. But his work received no serious attention for 34 years, till it was rediscovered by three botanists **Carl Correns** of Germany, **Hugo de Vries** of Holland and **Erich von Tschermak** of Austria in the year 1900. They studied the paper of Mendel while working independently on laws of inheritance and found results similar to those of Mendel. The copies of his paper were distributed in libraries in Europe and America and hence recognition was given to Mendel for his work in the field of genetics. Chromosome and the mechanism and role of meiosis were unknown at the time of Mendel. By predicting the gamete formation behaviour, he determined that inheritance does exist. The postulates of Mendel were accepted on the bases of the gene transmission from parent to offspring which is known as genetic transmission nowadays. The rediscovery of Mendel's work led to the progress in genetics studies of the twentieth century.

Non- Mendelian Inheritance

There are some patterns of inheritance in which characters do not segregate as per Mendel's laws. These non-mendelian inheritance patterns are seen in case of **Co-dominance**, where two alleles may be simultaneously expressed when both are present, rather than one fully determining the phenotype; **Incomplete dominance**, when two alleles may produce an intermediate phenotype when both are present, rather than one fully determining the phenotype; **Extra-chromosomal inheritance,** also known as **cytoplasmic inheritance** where genes present in the cytoplasm are inherited; sex- linked inheritance, where genes are carried by either of the sex chromosome; **Linkage**, which refers to the presence of two different genes on the same chromosome . Two genes that occur on the same chromosome are said to be linked, and these are transmitted together from one generation to next generation. All these patterns of inheritance do not follow Mendel's laws, therefore, their inheritance pattern is referred to as non-Mendelian inheritance These patterns of inheritance are described in detail in the following units.

This introductory chapter provides an overview of major developments in genetics in a chronological manner. It includes pre-mendelian theories and hypothesis, post-mendelian developments in genetics and the molecular era of genetics.

7.4 DEVELOPMENT OF GENETICS

7.4.1 Pre-Mendelian Genetics

Many scientists gave different theoretical views from time to time about the process of heredity before the rediscovery of Mendel's work in 1900. These views were varying differently and we will study these theories as **pre-Mendelian genetics**. Some of the major workers whose theories were slightly relevant to genetics are as follows: -

Pythagoras (580-500 B.C.) the Greek philosopher gave vapour theory. He proposed the idea that the new individual is formed by the combination of moist vapours that are produced from each organ of male body.

Hippocrates (400 B.C.) believed that reproductive material came from all parts of the body of a person, so the characters are directly handed down to the offspring.

Aristotle (350 B.C.), the famous Greek philosopher gave **fluid theory**. He believed that semen produced by man is purified blood and the menstrual fluid produced by the women is impure blood. These fluids combine at the time of mating. He also held that the semen of man provides vital ingredients for the formation of an embryo, female semen just provides the inert substance.

William Harvey (1578-1657) for the first time speculated that all animals arise from eggs and semen.

Swammerdam (1679) gave the **preformation theory** which suggested that a fully formed miniature adult called 'Homunculus" is already present inside the egg or sperm. This homunculus developed into an organism after fertilization. Human sperms were observed for the first time by Leeuwenhoek in 1677. Preformationist meant that only one parent inherited all traits— if the homunculus was present in the sperm then the traits were inherited from father or if present in the egg then from mother. The animalculists or spermists believed that homunculus was found in sperms, whereas the ovists thought that the ovum contained the preformed individual.

N Grew (1682) reported the reproductive parts of the plant for the first time.

Camerarious (1691 to 1694) described for the first time about the sexual reproduction in plants. Camerarious also worked to develop for the first time a plant hybrid by crossing hop plants and hemp with each other.

Thomas Fairchild (1717) performed an experiment and obtained artificial hybrid by cross-pollinating light red flowered Sweet Williams with pink flowered similar species of Sweet Williams and found out that characters of parents (mother and father) were developed in the offspring's. The new hybrid was called "Fairchild's Sweet William" or "Fairchild's mule".

This provided a means of artificial hybridization in plants. Similar work was performed by other workers on artificial pollinations between different related species and found somewhat similar results.

K.F. Wolff (1738-1794) rejected the preformation theory and gave the **theory of epigenesis**. This theory suggests that new body organs and tissues are formed step by step but not initially present in the early embryo. The concept for the theory of epigenesis was that the gametes contained undifferentiated living substances capable of forming organized body after fertilization.

Kolreuter (1761 to 1766) studied the process of pollination by making many crosses and found out the importance of insects for natural pollination. He was the first to carry out systematic reciprocal crosses in the period 1761-1766 and concluded that two parents contribute equally to the offspring's character. His work gave base to the subject that was much similar to that of Darwin and Mendel which was discussed hundred years later by both of them.

Jean-Baptiste Lamarck (1744-1829) proposed the theory of '**inheritance of acquired characters**' also called as **Lamarckism**. In the theory, he stated that only those organs of the body developed well which were in great use over long period of time and the organs which were in less or no use become reduced and finally disappeared. The theory was criticized by several workers because it failed to demonstrate the mechanism experimentally.

Charles Darwin (1809-1882) developed his theory of natural selection based on observations made during his expedition, which states that genetic differences can make individuals suited for their environment. He published his theories in book *Origin of Species* in 1859. Darwin tried to explain the mechanism of natural selection by giving physical basis of heredity in the **theory of pangenesis** in 1868. He suggested that every body part produced minute particles called as 'gemmules' or 'pangenes' which are transported through bloodstream to sex organs and assemble there as gametes. During fertilization, gemmules from both parents are mixed which lead to development of mixture of maternal and paternal organs and tissues in progeny. The hereditary characteristics are thus believed to be transmitted from the parents to offspring. The concept of pangenesis theory was discarded by Francis Galton. To test the validity of pangenesis, he performed a series of blood transfusion experiment on different pigmented rabbits. He establishes no proof for the existence of gemmules.

John Knight (1799) and **John Goss (1824)** carried out breeding experiments on pea plant using two varieties- unpigmented and pigmented. The results of their experiment were similar to those obtained by Mendel about forty years later. But they failed to formulate the laws of inheritance because they could not give mathematical treatment to data. Moreover, main aim of performing these experiments was improvement of the varieties rather than understanding the mechanism of heredity.

M. J. Schleiden and T Schwann (1839) gave the "**cell theory**" which stated that all living organisms are composed of one or more cells and the cell is the most basic unit of life. Third

view was added to this theory by **Rudolf Virchow** in 1855, that all cells arise from pre-existing cells only- *Omnis cellulae cellula* in Latin.

Gregor Johann Mendel (1822-1884), gave his famous postulates on inheritance of traits on the basis of his work on garden pea. Mendel's work was overlooked for 34 years until it was rediscovered by three botanists- **Carl Correns** of Germany, **Hugo de Vries** of Holland and **Erich von Tschermak** of Austria in 1900.

7.4.2 Post Mendelian Developments in Genetics

Friedrich Miescher (1871) was the first researcher to isolate nucleic acid from nucleus of white blood cells which he called *nuclein.* Later he raised the idea that the nucleic acids could be involved in heredity.

Walter Flemming (1879) studied the animal cell division and described the chromosome behaviour in it. He explained the whole process of mitosis by staining the chromosome and observing it in 1882.

August Weismann (1834–1914), a German biologist, gave the **germplasm theory** to explain reproduction in animals. His theory holds that the cells in the reproductive organs carry a complete set of genetic information that is passed to the egg and sperm. He distinguished two types of tissues- somatoplasm and germplasm. Somatoplasm is necessary for proper functioning and survival of the organism, but does not contribute to sexual reproduction; whereas, the germplasm produces the gametes which lead to sexual reproduction. Weismann finally proved that acquired characters are not inherited through his experiment on mice. He cut off the tails of mice for 22 consecutive generations but always got tailed mice. His demonstration disposed off the hypothesis of pangenesis and inheritance of acquired characters.

Hugo de Vries (1901), a Dutch botanist, who is one of the independent rediscoverer of Mendelism, said about the formation of new hypothesis in 1901. He proposed the theory of mutation. This theory was based on his observations on evening primrose, *Oenothera lamarkiana*. He studied this plant for many years in wild forms and saw some instinctive changes in some of these wild plants. These plants relay on the stem height, the color of the flowers and the size of the leaves, color and leaves shapes. They saw that these changes were necessary and after all, many new varieties were born. He succeeded in cultivating all these new varieties and nominated them as mutant varieties.

Walter Sutton and Theodore Boveri (1903) formulated the **chromosome theory of inheritance**. They studied the chromosomes during meiosis and observed that their segregation during meiosis and segregation pattern of Mendel's genes during gamete formation were similar which suggested that Mendelian factors or genes are carried on chromosomes.

William Bateson (1905) proposed the term 'genetics' (a Greek word meaning to generate). Genetics is the branch of biological science which deals with heredity and variations. This

branch deals generally with the laws governing the transmission of hereditary potentialities from parents to offspring's.

Friedrich Laibach (1907) suggested for the first time of Arabidopsis as a model organism in 1907. It was a notable work on Arabidopsis which was performed and published in 1907. When Friedrich Laibach, a student in Strasburger's laboratory in Bonn, published an account of the chromosome number of several plants. He was attempting to find a plant with a small number of large chromosomes to be used in experiments to determine the individuality of chromosomes.

Wilhelm Johannsen (1909) coined the term "**gene**" to explain mendelian unit of heredity. He also brought up the term genotype and phenotype to distinguish between the genetic traits and outward appearance of an individual. According to him the genotype was the sum total of heredity of an individual, while phenotype represents the observable structural and functional properties of an individual which are produced by the interaction between genotype and environment.

Thomas Hunt Morgan (1910) proposed the theory of **sex-linkage**. He confirmed the chromosome theory of inheritance with his white-eyed fruit fly. Normally *Drosophila melanogaster* have red eyes, but Morgan's new fly had white eyes and his experiments showed that inheritance of white eyes is linked to x-chromosome. Therefore, the discovery that chromosomes carry genes and inheritance of a specific trait is linked to a particular chromosome was discovered.

B.O. Dodge (1927) studied sexual reproduction for the first time in *Neurospora* and used it as a genetic organism (17). *Neurospora* was later used by **G. W. Beadle** and **E. L. Tatum (1941)** in X-ray mutation experiments. The results of their experiments led them to **one gene-one enzyme hypothesis**, in which they postulated that genes express themselves by synthesis of enzymes.

H. J. Muller (1927) demonstrated X-rays induced mutations in *Drosophila* for the first time.

L. J. Stadler (1928) demonstrated mutagenic effects of X-rays in barley and later in maize also.

Frederick Griffith (1928) performed experiments using two strains of bacteria *Streptococcus pneumoniae* in mice. This experiment led to the discovery that bacteria are capable of transferring genetic information through a process known as **transformation**. He took a type III-S (smooth) which was virulent, and a type II-R (rough) strain which was nonvirulent. When injected into a live mouse, the type-III S bacteria multiplied within the mouse's bloodstream and ultimately killed the mouse. On the other hand, when type-II R bacteria were injected into a mouse, the mouse stayed alive. To confirm that the proliferation of the smooth bacteria was the main cause of the death of mouse, Griffith gave heat treatment to smooth bacteria and killed it before injecting it into the mouse. Mouse survived in that case. The live type-II R bacteria when mixed with heat-killed type-III S bacteria, caused mouse death. From this unexpected result he concluded that there is something which

transformed from heat killed R- strain to live R-strain. He showed that some genetic material from the dead bacteria had been transferred to the living bacteria and provided them with a new trait.

Avery, MacLeod, McCarty (1944) proved that DNA is the genetic material. These three scientists confirmed Griffith's findings and tried to find out the transforming material by adding additional biochemical techniques into their experiment. During this time, the researcher already knew that DNA, RNA, Proteins and Carbohydrates are main constituents of living cells. They prepared bacterial extracts from type-III S strains containing each of these molecules. After many attempts with different types of extracts, scientists discovered that the extract which contained purified DNA was the only one able to convert the type R bacteria into type S bacteria.

Alfred Hershey and **Martha Chase (1952)** demonstrated and confirmed that DNA is genetic material. The experiments that were conducted by Avery and his colleagues were perfect, but many scientists didn't accept DNA as the genetic material. An experiment was carried out by Hershey and Chase using the phage (virus) T_2. They gave the reason that phage infection must require the introduction into the bacterium of the specific information that dictates viral reproduction. Phosphorus is not found in proteins but is an integral part of DNA; conversely, sulphur is present in proteins but never in DNA. Hershey and Chase incorporated the radioisotope of phosphorus (^{32}P) into phage DNA and that of sulphur (^{35}S) into the proteins of a separate phage culture. They then used each phage culture independently to infect *E. coli* with many virus particles per cell. After sufficient time for injection to take place, they used centrifugation to separate the bacterial cells from the phage ghosts and then measured the radioactivity in the two fractions. When the ^{32}P-labeled phages were used, most of the radioactivity ended up inside the bacterial cells, indicating that the phage DNA entered the cells. ^{32}P can also be recovered from phage progeny. When the ^{35}S-labeled phages were used, most of the radioactive material ended up in the phage ghosts, indicating that the phage protein never entered the bacterial cell. This experiment confirmed that DNA is the hereditary material.

7.4.3 Beginning of the Era of DNA and Molecular Genetics

J.D. Watson and F.H.C. Crick (1953) defined the structure of DNA. They stated that DNA is a double helical structure with two strands of DNA joined by hydrogen bonds and running in opposite direction so the structure is ladder and always consist of A, T and G, C base pairs. DNA was also studied by X-ray crystallography by **Maurice Wilkins** which demonstrated that DNA helix has a diameter of 20Å and one round of the helix is of about 34 Å. Wilkins, Watson and Crick were awarded a Nobel Prize in 1962 together for their work on the structure of DNA.

Arthur Kornberg (1956) isolated the first DNA polymerizing enzyme, now known as DNA polymerase I. This won him the Nobel Prize jointly with **Severo Ochoa** in 1959 for their discovery of the mechanisms in the biological synthesis of RNA and DNA.

Vernon Ingram (1956) and his co-workers determined that a particular chemical alteration in the haemoglobin proteins is the root cause for the disease sickle cell anaemia. Therefore, a new discovery that the alteration in a single amino acid in a protein can cause disease was traced.

Francis Crick (1957) proposed the **central dogma of molecular biology**. This was for the first time a clear explanation was given for the link between the DNA molecule sequence and proteins production. Francis Crick in 1958 predicted the existence of tRNA when he suggests that amino acids are brought to a template mRNA by a nucleic acid adaptor molecule (tRNA) and, these molecules actually fits on the mRNA.

Matthew Meselson and Franklin Stahl (1958) performed an experiment and found that DNA can replicate semi conservatively. Each strand from the parent DNA molecule ends up paired with the new strand from the daughter generation. So, the "Semi-conservative Replication of DNA" was demonstrated.

Jerome Lejeune (1959) and his colleagues discovered that trisomy in 21^{st} pair of chromosome is the cause for the down's syndrome disease. The three pair of chromosome instead of two is the root cause of disease and this extra chromosome interferes with the normal development. So the chromosome abnormality was discovered.

F. Jacob and J. Monod (1961) proposed the **lac operon** model of gene regulation in bacteria based on their study of the genes in *E. coli* that code for enzymes that affect the breakdown of **lactose**.

Sydney Brenner, Francis Jacob and Mathew Meselson (1961) discovered that mRNA takes the genetic information from the DNA of nucleus to the protein making machinery called ribosomes in cytoplasm. Therefore, it was found that mRNA transfers information.

Marshall Nirenberg (1966) and other colleagues found out genetic code that allows the nucleic acids with their four letter alphabet to determine the order of 20 amino acids in proteins and hence genetic code was cracked for the first time.

Margaret Dayhoff (1965) published for the first time "Atlas of Protein Sequence and Structure". It contained the sequence of 65 proteins and was considered the first publication in bioinformatics. The one-letter code used for amino acids was also developed by her.

Waclaw Szybalski and W. Summers (1967) showed that out of the two strands that make up the DNA molecule, only one is used during transcription.

Discovery of **DNA ligases (1967)** in the laboratories of Gellert, Lehman, Richardson and Hurwitz was a very important event in molecular biology.

In the late 1960s and early 1970s, molecular biologists **Werner Arber**, **Hamilton O. Smith**, and **Daniel Nathans** discovered and explained **restriction enzymes** which identifies and cut particular short sequence of DNA. The fragments that were obtained can be used up for the

analysis of DNA. These enzymes in future were used as tool for mapping genomes and recombinant DNA technology.

Howard Temin and **David Baltimore (1970)** discovered **reverse transcriptase** enzyme.

Paul Berg (1972) created **first recombinant DNA** molecule by using DNA from different species of organisms namely – SV40 monkey virus and a bacterial virus known as lambda bacteriophage and joining these with the help of DNA ligase enzyme. This hybrid DNA could be inserted into a host cell where it replicates.

Herbert Boyer and **Stanley N. Cohen (1973)** carried out first animal gene cloning by fusing a gene from the African clawed frog *Xenopus laevis* with DNA from the bacterium *E. coli* and introducing this recombinant DNA back into and *Escherichia coli* cell. There, the frog DNA was copied and the gene it contained directed the production of a specific frog protein.

In **1975,** two groups of researchers- one was of **Frederick Sanger** and colleagues, and another was of **Alan Maxam and Walter Gilbert**, they both developed methods for **DNA sequencing**.

Herbert Boyer (1976) founded Genentech, the first genetic engineering company. This company made the first human protein in a bacterium and by 1982 first recombinant DNA drug was marketed that was Human Insulin.

Richard Roberts and Phil Sharp (1977) discovered **introns** in eukaryotic genes. These are non-coding regions in a gene that do not directly specify the amino acids that make protein products.

David Botstein (1978) described the use of **RFLP** (restriction fragment length polymorphism) technique in genetic mapping to indicate genetic differences among individuals. RFLPs are variations in DNA sequence that can be observed by digesting DNA with restriction enzymes.

In **1981**, the **first transgenic mice and fruit flies** were developed. These transgenic animals gave a new way to test the function of genes.

Kary Mullis (1983) invented the polymerase chain reaction (**PCR**). This technique is used to rapidly amplify i.e. make billions of copies of a specific segment of DNA within few minutes.

Leroy Hood (1986) developed **automated sequencing machine** for fast and cost effective sequencing of DNA.

In **1987, First human genetic map** was made which was based on 400 restriction fragment length polymorphisms (RFLPs). This type of maps is generally useful in locating the genes that are responsible for diseases.

In **1987, Yeast artificial chromosomes** (YAC) were discovered by David Burke. Scientists discovered that yeast artificial chromosomes can carry larger fragments of DNA as compared

to plasmids and viruses. Human genome mapping was made easier because of the ability of YACs in dealing with much larger pieces of DNA.

In **1989,** repetitive DNA sequences called **Microsatellites** were discovered as the new genetic markers. These are used as genetic landmarks to distinguish between people because they are scattered throughout the genome and can be detected easily using the polymerase chain reaction (PCR). Another DNA marker called sequence tagged sites (STS) detectable by PCR can be used to make physical maps of human chromosomes.

In **1990, Human Genome Project** was launched by the US Department of Energy and National Institute of Health with aim to map and sequence the human genome. This was proposed to be a 15-year project.

In **1994,** First genetically modified food was discovered which was **Flavr Savr tomato**. It was modified to stay firm after harvesting so that they do not over ripe during transportation. It was approved by the Food and Drug Administration for sale.

In **1995, first complete genome was sequenced**, that was of bacterium *Haemophilus influenza.* Later in this year, researchers sequenced the smallest known genome, that of the bacterium *Mycoplasma genitalium*.

In **1996,** the yeast *Saccharomyces cerevisiae* genome was sequenced. This was the first complete genome sequence of a eukaryote.

In **1997,** *Escherichia coli* genome was sequenced. With the help of *E. coli* extensive study of bacterium could be done by scientists.

In **1998,** sequencing of genome of roundworm *Caenorhabditis elegans* was completed. It was the first genome sequence of a multicellular organism.

In **1999,** a first full length sequence of a human chromosome (Chromosome no. 22) was produced under the human genome project.

In **2001, t**he first draft of human genome sequence was released which covered about 90% of the human genome.

In **2002,** mouse genome was sequenced and published by The International Mouse Genome Sequencing Consortium. This consortium was part of human genome project.

In 2002, rice genome was sequenced by two groups of researchers.

In **2003,** the sequencing for the human genome was completed by the International Human Genome Sequencing Consortium. This sequence covers 99 % of the human genome and is 99 .99% accurate.

In **2016** a genome is sequenced in outer space for the first time with NASA astronaut Kate Rubins using a MinION device on board the International Space Station.

7.5 SUMMARY

In this unit, we have discussed the meaning of genetics, important hypothesis and works of pre-Mendelian and post-Mendelian genetics. We also learnt how the field of genetics developed from Mendelian genetics to molecular genetics. So, let us sum it up in key points:

1. Genetics is the study of heredity and variation.
2. Heredity includes those traits or characters which are transmitted from generation to generation.
3. Variations are the differences between the parents and the progeny and among the progeny. These variations are due to sexual reproduction.
4. Gregor John Mendel is regarded as the Father of Genetics. He performed experiments on garden pea and gave his famous postulates of – unit factors; dominance; segregation; and independent assortment.
5. Mendel's work remained unrecognised until 20^{th} century when Carl Correns, Hugo de Vries and Eric von Tschermak rediscovered his work.
6. Major theories of the pre- Mendelian time includes- preformation theory, theory of epigenesis, theory of inheritance of acquired characters, theory of pangenesis, cell theory etc.
7. Post-Mendelian developments include isolation of nucleic acid from white blood cells by F. Miescher, revelation of details of mitosis by W. Flemming, study of sexual reproduction for the first time in *Neurospora* and its use as a genetic organism by Dodge (1927). Later, *Neurospora* was used for x-ray mutation experiments by Beadle and Tatum which led them to develop one gene-one enzyme hypothesis (1941).
8. Some important theories of post mendelian era are: germplasm theory, chromosome theory of inheritance and, theory of sex linkage.
9. Griffith's famous transformation experiment (1928) led to the discovery that bacteria are capable of transferring genetic information from dead bacteria to living bacteria and give them a new trait.
10. Experiments of Avery, MacLeod and McCarty (1944) and Hershey and Chase (1952) proved that DNA is the genetic material. This great discovery led to the era of DNA and molecular genetics.
11. Watson and Crick (1953) gave the double helical structure of DNA. They along with Wilkins were awarded the Nobel Prize in 1962 together for their work on the structure of DNA.
12. Discoveries that led to the development of molecular era of genetics were- DNA polymerase I, central dogma of molecular biology, semi-conservative replication of DNA, lac operon model of gene regulation in bacteria and, genetic code.
13. Discovery of DNA ligases and restriction enzymes were very important events in molecular biology. These enzymes were later used as tools for recombinant DNA technology.
14. First recombinant DNA molecule was obtained in 1972 by Paul Berg using DNA from SV40 monkey virus and lambda bacteriophage.

15. First animal gene cloning was carried out in 1973 by Boyer and Cohen using a gene from African clawed frog and *E. coli* DNA.
16. One of the most important discoveries of molecular era was the methods of DNA sequencing developed separately by Frederick Sanger and colleagues; and Alan Maxam and Walter Gilbert.
17. First transgenic mice and fruit flies were developed in 1981 which gave a new way to test the function of genes.
18. Other major discoveries and inventions include- Polymerase Chain Reaction, yeast artificial chromosome, genetic markers, automated sequencing machine, etc.
19. First human genetic map was made in 1987 using RFLPs.
20. Human Genome Project was launched in 1990 and it was completed in 2003.
21. First genetically modified food was developed in 1992 which was Flavr Savr tomato.
22. Complete genomes of many organisms were sequenced 1995 onwards which include bacteria *Haemophilus influenza*, *Mycoplasma genitalium*, yeast, *E. coli*, roundworm *Caenorhabditis elegans*, mouse, rice, etc.
23. In 2016, a genome has been sequenced in outer space by NASA astronaut Kate Rubins using a MinION device.

7.6 *GLOSSARY*

Bioinformatics: The science of collecting and analysing complex biological data such as genetic codes.

Conjugation: Process by which one bacterium transfers genetic material to another through direct contact.

DNA sequencing: Process of determining the exact sequence of nucleotides within a DNA molecule.

Gene cloning: The production of exact copies or clones of a particular gene or DNA sequence using genetic engineering techniques.

Genes: A gene is a region of DNA that encodes a protein.

Genetic map: A graphic representation of the arrangement of a gene or a DNA sequence on a chromosome.

Genome: The complete set of genetic material in an organism.

Genotype: The genotype is the set of genes in DNA which is responsible for a particular trait.

Hybrid: Offspring of two individuals of different breeds, varieties, species or genera through sexual reproduction.

Offspring: Children or young born of living organisms, also known as progeny

Phenotype: The physical expression or characteristics of a particular trait.

Plasmid: A small, circular, extra-chromosomal DNA molecule within a bacterial cell that can replicate independently.

Proliferation: A rapid increase in growth or production of cells by multiplication of parts.

Radioisotope: A radioactive isotope of a chemical element.

Recombinant DNA: A DNA produced by combining genetic material from two or more different sources by means of genetic engineering.
Trait: Any genetically determined characteristic or feature of an organism.
Transcription: Process by which the information in a strand of DNA is copied into a new molecule of messenger RNA.
Transformation: Genetic alteration of a cell by the direct uptake and expression of DNA from its surroundings.
Transgenic: An organism containing genes from another organism put into its genome through recombinant DNA techniques.
Virulence: The ability of a pathogenic organism to cause disease.
X-ray crystallography: A tool used for determining the atomic and molecular structure of a crystal.

7.7 SELF ASSESSMENT QUESTION

7.7.1 Very short answer questions

1. Who coined the term genetics?
2. Name the plant on which Mendel performed his experiments.
3. Who reported reproductive parts of the plant for the first time?
4. Who proposed the theory of epigenesis?
5. Which theory was proposed by Lamarck?
6. Who gave cell theory?
7. Who isolated nucleic acid for the first time?
8. Which theory suggested that 'Homunculus' was already present inside the egg or sperm.
9. Who developed artificial hybrid for the first time?
10. Name the theory given by Schleiden and Schwann in 1839.
11. Name the workers who rediscovered Mendel's work.
12. Name the theory which suggests presence of somatoplasm and germplasm in an organism.
13. Who gave the chromosomal theory of inheritance?
14. Name the bacteria used by Griffith for his transformation experiments?
15. Name the scientists who proposed the central dogma of molecular biology.
16. Who gave the Lac operon model of gene regulation in bacteria?
17. Write full form of PCR.
18. In which year Human Genome Project was launched.
19. Name the first genetically modified food.
20. Name the organism whose complete genome was sequenced for the first time.
21. What phenotypic ratio is obtained in Mendel's Law of segregation?

7.7.1-Answer key: 1. William Bateson, 2. *Pisum sativum*, 3. N. Grew, 4. K.W. Wolff, 5. Inheritance of acquired characters, 6. Schleiden & Schwann, 7. Friederich Miescher, 8. Preformation theory, 9. Camerarious, 10. Cell theory, 11.Carl Correns, Hugo de Vries & Eric von Tschermak, 12. Germplasm theory, 13. Sutton & Boveri, 14. Streptococcus pneumonia,

15. Francis Crick, 16. Jacob & Monod, 17. Polymerase Chain Reaction, 18. 1990, 19. Flavr Savr Tomato, 20. *Haemophilus influenza*, 21. 3:1

7.7.2 Multiple Choice Questions

1. Mendel's work was rediscovered by

(a) Walter Sutton and Theodor Boveri
(b) John Goss
(c) M. Schleiden and T. Schwann
(d) C. Correns, H. Vries & E. V. Tschermak

2. Which of the following workers isolated nucleic acid from cells for the first time?

(a) Friedrick Miescher
(b) Hugo de Vries
(c) Walter Flemming
(d) William Bateson

3. August Weismann's theory which state that the cells in the reproductive organs carry a complete set of genetic information that is passed to egg and sperm is known as

(a) Chromosomal theory of inheritance
(b) Theory of pangenesis
(c) Theory of epigenesis
(d) Germplasm theory

4. The term 'genetics' was coined by

(a) William Bateson
(b) T.H. Morgan
(c) B.O. Dodge
(d) None of the above

5. T.H. Morgan is known for

(a) Chromosomal theory of inheritance
(b) Cell theory
(c) Theory of sex linkage
(d) One gene - one enzyme hypothesis

6. X-ray induced mutations in *Drosophila* were demonstrated for the first time by

(a) H.J. Muller
(b) W. Johannsen
(c) T.H. Morgan
(d) F. Laibach

7. Beadle and Tatum's one gene-one enzyme hypothesis was based on their X-ray mutation experiments on

(a) *Drosophila*
(b) *Neurospora*
(c) *Streptococcus*
(d) None of the above

8. Which of the following workers proved that DNA is the genetic material?

(a) Griffth
(b) Stadler
(c) Hershey and Chase
(d) Avery, Macleod and Mc Carty

9. Nobel prize in 1962 for work on structure of DNA was given to

(a) Watson and Crick
(b) Wilkins, Watson and Crick
(c) Maurice Wilkins
(d) Jacob and Wool man

10. Central dogma of molecular biology was proposed by

(a) Francis Crick
(b) F. Jacob
(c) Arthur Kornberg
(d) Nome of the above

11. Semi conservative replication of DNA was demonstrated by

(a) Jacob and Monad (b) Watson and Crick
(c) J. H. Tjio and A. Levan (d) Meselson and Stahl

12. Jacob and Monod's work on gene regulation in bacteria led them to propose
(a) Lac Operon model (b) Central dogma of molecular biology
(c) Both a and b (d) None of the above

13. Genetic code was cracked for the first time by
(a) M. Nirenberg (b) D. Baltimore
(c) H.G. Khorana (d) None of the above

14. Reverse transcriptase enzyme was discovered by
(a) Temin and Baltimore (b) Boyer and Cohen
(c) Kimura (d) None of the above

15. DNA sequencing methods were developed by
(a) Frederick Sanger (b) Alan Maxam and Walter Gilbert
(c) Both a and b (d) None of the above

16. PCR was discovered by
(a) Leroy Hood (b) Karry Mullis
(c) David Botstein (d) Herber Boyer

17. First genetically modified food was
(a) Tomato (b) Pear
(c) Strawberry (d) Banana

18. DNA polymerase enzyme was first isolated by
(a) Boyer & Stanley N Cohen (b) Kary B. Mullis
(c) Arthur Kornberg (d) None of the above

19. The phenotypic ratio obtained in Mendel's dihybrid cross was
(a) 3:1 (b) 15:1
(c) 9:3:3:1 (d) None of the above

20. The observable physical characters of a plant is called as
(a) Genotype (b) Dominant
(c) Phenotype (d) Recessive

7.7.2 Answer key: 1.d, 2.a, 3.d, 4.a, 5.c, 6.a, 7.b, 8.d, 9.b, 10.a, 11.d, 12.a, 13.a, 14.a, 15.c, 16.b, 17.a, 18.c, 19.c, 20.c

7.7.3 Fill up the following blanks:

1-The title of Mendel's paper was _____.
2- Walter Sutton and Theodor Boveri are known for their _____.
3-The term 'gene' was coined by_____.
4- Charles Darwin gave the theory of _____.
5- Germplasm theory was given by_____ .

6- Nucleic acid was isolated for the first time from _____.
7- Sexual reproduction in plants was described by _____.
8- The first artificial hybrid was made by _____.
9- Theory of pangenesis by given by _____.
10- Mendel performed his experiment on pea at _____.
11- Mitosis was explained by _____.
12- Thomas Hunt Morgan proposed the theory of _____.
13- Double helical structure of DNA was given by_____.
14- Enzyme that identifies and cut particular short segment of DNA are known as___.
15- First recombined DNA molecule was created by _____.
16- YAC stands for _____.
17- Smallest known genome sequenced so far is of _____ .
18- The first completely sequenced eukaryotic genome is
19- Sequencing of human genome was completed in_____.
20- The device used for sequencing a genome in outer space is known as _____.
21- The non-coding regions of a gene are known as _____.

7.7.3 Answer Key: 1. Experiments in Plant Hybridization, 2. Chromosome theory of inheritance, 3. Johansen, 4. Pangenesis, 5. August Weismann, 6. white blood cells, 7. Camerarius, 8. Thomas Fairchild, 9. Charles Darwin, 10. Augustinian Monestry Brunn, 11. Walter Flemming, 12. Sex linkage, 13. Watson & Crick, 14. Restriction enzyme, 15. Paul Berg, 16. Yeast Artificial Chromosome, 17. *Mycoplasma genitalium*, 18. Yeast, 19. 2003, 20. MinION device, 21. Introns

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7.9 SUGGESTED READINGS

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7.10 TERMINAL QUESTIONS

7.10.1 Long Answer type questions

1. Describe the major theories of heredity given before rediscovery of Mendel's work.
2. Give a brief account of Mendel and his work.
3. Briefly describe the experiments which lead to the discovery that DNA is the genetic material.
4. Describe the major discoveries of molecular era of genetics.

UNIT-8 EXTRACHROMOSOMAL INHERITANCE AND CYTOPLASMIC MALE STERILITY

8.1 OBJECTIVES

After reading this unit students will be able-

1. To study extra chromosomal inheritance in plants
2. To study extra nuclear inheritance by organelles
3. To study male sterility in plants

8.2 INTRODUCTION

All genetic loci are located on the chromosomes in the cell nucleus. The genes of nuclear chromosomes have a significant and key role in the inheritance of almost all traits from generations to generations. Nuclear chromosomes altogether cannot be considered as the sole vehicles of inheritance because certain experimental evidences suggest the occurrence of certain extra nuclear genes or DNA molecules in the cytoplasm of many prokaryotic and eukaryotic cells. These genes are found inside the organelles, such as mitochondria and chloroplasts in the cytoplasm. These organelles have a circular DNA molecule similar to that of a eukaryotic system. Extra nuclear inheritance or cytoplasmic inheritance is the transmission of genes that occur outside the nucleus. The characteristics inherited by them are known as extra chromosomal inheritance or cytoplasmic inheritance.

Evidences for cytoplasmic factors

Traits with extra nuclear basis are identified by the accumulated evidence from a number of diagnostic criteria such as follows:

1. Female gamete contributes almost all of the cytoplasm to the zygote and male gamete (sperm or pollen) contributes only a nucleus, an inheritance pattern that differs between reciprocal crosses suggests a cytoplasmic involvement. This is clearly the basis for uniparental or maternal inheritance where the progeny always resemble one parent, most commonly the female parent (e.g., shell coiling in *Limmaea peregra*).
2. Differences in reciprocal crosses which cannot be attributed to sex- linkage or some other chromosomal basis tend to implicate extra nuclear factors (e.g., chloroplast inheritance in *Mirabilis jalapa*).
3. The uniparental inheritance of a trait which cannot be attributed to unequal cytoplasmic contributions from parental gametes may, however, involve cytoplasmic factors (e.g., Streptomycin resistance in *Chlamydomonas*).
4. Trait fails to exhibit linkage to any known nuclear linkage groups and assort independently from nuclear genes, a cytoplasmic mode of inheritance is suggested.
5. Many types of mutants that fit the above criteria will show segregation during mitotic division.
6. The acquisition of traits or conditions controlled by self replicating substances within the cytoplasm, such as mitochondria or chloroplasts.

The cytoplasmic inheritance, therefore, will be understood to be based on cytoplasmically located, independent, self-replicating nucleic acids, which differ from chromosomal genes by their location within the cell, and have their own nucleotide sequences. The smallest heritable extra chromosomal unit is called a plasmagene. All of the plasmagenes of a cell constitute the Plasmon.

8.2.1 Characteristics and Detection of Cytoplasmic Inheritance:

Cytoplasmic inheritance does not show Mendelian inheritance. Here, a trait is transmitted from the parent to offspring through nonchromosomal, cytoplasmic means. Additional pattern of inheritance that deviate from a Mendelian pattern include certain special features.

I. Lack of Mendelian segregation and typical Mendelian ratios: Hereditary traits which are transmitted by cytoplasm do not show Mendelian segregation in crosses and in reciprocal crosses with respect to a particular set of characteristics controlled by a set of cytoplasmic genes producing dissimilar hybrids.

II. Maternal inheritance: Maternal inheritance is characterized by an extra-chromosomal inheritance that persists for many generations. The extra-chromosomal material may be from either the mitochondria or the chloroplast. The patterns of inheritance are not associated with meiosis or mitosis because the organelles are in the cytoplasm not in the nucleus. Organelles have circular chromosomes. Maternal (uniparental) effects are strictly defined as changes that occur only in the first generation of progeny.

Maternal inheritance is characterized by an extra chromosomal inheritance i.e., cytoplasmic factors that are transmitted to the succeeding generation through the egg of female organism. The female phenotype in a cross is always expressed in its offspring. In higher plants and animals, ovum or egg cell is comparatively large and contains large amount of cytoplasm. But male gametes or sperms have very little amount of cytoplasm. So, under this situation, most of cytoplasmic factors are transmitted to the progeny through the ovum of mother. It is known as maternal inheritance or trans-ovarian transmission. In this mode of transmission, all the offspring's of the parents have maternal condition and only female progeny can transmit the cytoplasmic characteristics to the succeeding generations.

In certain cases, it has been observed that certain characteristic phenotypic traits of F_1, F_2 and F_3 progeny are not the expression of their own genes, but rather those of the maternal parents. Such phenotypic expressions of maternal genes (genotype) may be short-lived or may persist throughout the life-span of the individual. The substances which produce the maternal effects in the progeny are found to be transcriptional products (i.e., mRNA, rRNA and tRNA) of maternal genes which have been manufactured during oogenesis and, which exist in the ooplasm of unfertilized eggs in the form of inactive protein coated and late translating mRNA molecules (informosomes) or inactivated rRNA and tRNA. These transcriptional products of maternal genes produce their phenotypic effects during early cleavage and blastulation when there occur

little or no transcription since; maternal and paternal genes of zygote remain engaged in mitotic replication or duplication of DNA.

8.3 STUDY OF EXTRA-NUCLEAR INHERITANCE BY CELLULAR ORGANELLES

The eukaryotic cells possess a complement of chromosomes in the nucleus and extra DNA molecules or chromosomes in their mitochondria and chloroplasts. Mitochondria, chloroplasts, endosymbionts and cellular surfaces contain naked circular DNA and protein synthesizing apparatus which does not resemble with that of genes of nuclear chromosomes and is known by different terms such as extra-chromosomal, cytoplasmic or extra-nuclear inheritance.

These extra nuclear genetic materials present in the organelles are autonomous and code only for limited number of enzymes and polypeptides. Certain enzymes required for cellular respiration are synthesised in the mitochondria.

Similarly, chlorophyll and other pigments are synthesised in the plastid. Besides the involvement of such biosynthetic activities, these organelles DNAs are directly associated with the inheritance of some phenotypes which are not controlled by the nuclear genes.

The genetic material of chloroplasts and mitochondria are transmitted via the egg. During fertilization only the nucleus of the male gamete enters the egg, leaving the cytoplasm outside. Thus, the cytoplasm or the cytoplasmic genes of the zygote are contributed only by the egg and not by the male gamete. Therefore, the extra chromosomal inheritance is also known as maternal inheritance.

8.3.1 Endosymbiotic origin of Chloroplasts and mitochondria

Free living prokaryotes ancestors of chloroplasts and mitochondria invaded plant and animal cells but provide useful function and so a symbiotic relationship developed over time. Chloroplasts and mitochondria are organelles that contain their own DNA and protein-synthesizing apparatus. A widely held theory concerning their origin proposes that they were once infectious endosymbiotic prokaryotes that evolved such a dependence on the gene products of the host that they are no longer able to function autonomously (Fig 8.1).

This theory has been supported by the fact that the genetic components of these organelles are often similar to those found in prokaryotes. For example, the chloroplasts of certain algae and *Euglena* contain 70S type small ribosomes and “naked” chromosomes or DNA which is circular. Their protein synthesis begins with the amino acid N-formyl methionine, as does prokaryotic protein synthesis, and their DNA-dependent RNA polymerase is sensitive to the inhibitor rifampicin. The genetic materials of chloroplasts and mitochondria will be transmitted to offspring almost exclusively through the egg.

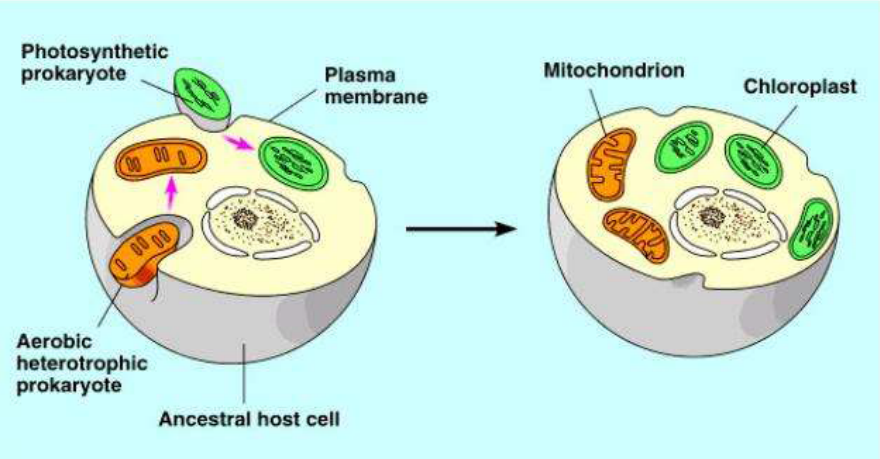


Fig 8.1: Endosymbiotic origin of Mitochondria and Chloroplast

8.3.1.1 Inheritance of chloroplasts:

The cytoplasm of plants bears minute pigments called chloroplast. Chloroplast genomes are 130-150 kb in size. Most genes are involved in photosynthesis. Corn has 20-40 chloroplasts per cell; with each chloroplast having 20-40 chromosomes (can make up 15% of DNA). Chloroplast arises from smaller DNA particles of the ovum and multiplies by division. During the formation of gamete a chloroplast enters the egg and the pollen receives none. Thus chloroplast is entirely a maternal contribution.

1. Chloroplast inheritance in Four O'clock plant (*Mirabilis jalapa*)

Chloroplast inheritance means the inheritance of chloroplast characteristics due to plasma genes located in chloroplast. Cytoplasmic inheritance or maternal inheritance or extra-chromosomal inheritance of chloroplast has been studied in Four-o'clock plant, *Mirabilis jalapa* by Corens (1908). Albomaculatus race of this plant have variegated leaves with white and green patches. Sometimes a branch is formed which bears leaves which are either green or white or variegated. In contrast to other higher plants, mirabilis contains three types of leaves and parts: (1) full green leaves or branches having chloroplast, (2) white (pale) leaves or branches having no chloroplast, (3) variegated branches having leucoplast in white (pale) areas and chloroplast in green patches (Fig 2). Thus it forms the mosaic pattern of coloration on a leaf. Due to certain inheritable defects chloroplast of all cells or some cells of leaf often are unable to synthesize the chlorophyll pigments. Such cells remain non- green and form white or yellow coloured leaf, or white or yellow patches, interspersed with areas containing normal green cells with healthy chloroplasts.

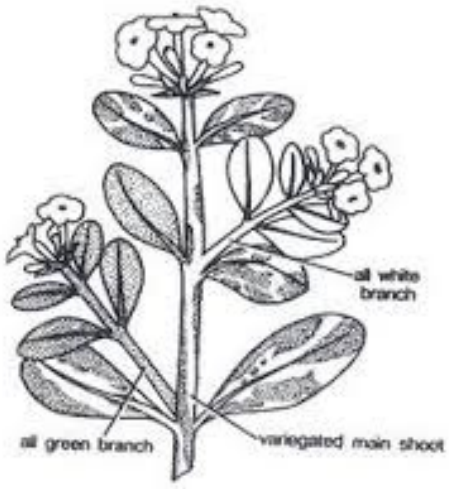


Fig 8.2: Leaf variegation in four O' clock plant (Mirabils jalapa)

Because, the chlorophyll pigment of chloroplast is related with photosynthesis of food and leucoplasts are incapable to perform photosynthesis, so the white or pale parts of plant survive by receiving nourishment from green parts. Correns reported that flowers on green branches produced only green offspring, regardless of the genotype and phenotype of pollen parent and likewise, flowers from the white or pale branches produced only white or pale seedlings regardless of genotype and phenotype of pollen parent. The plants developing from the white or pale seedlings die because they lack chlorophyll and cannot carry on photosynthesis. Crosses were made among the flowers associated with each leaf colour (Table 1). Corens (1908) further reported that flowers from the variegated branches yielded mixed progeny of green, white (pale) and variegated plants in widely varying ratios. These results are summarized in Table 1.

TABLE 1. Chloroplast inheritance in variegated four o'clock plants

S.No.	Branch of origin of the male parent	Branch of origin of the female parent	Progeny
1.	Green	Green, Pale or white Variegated	Green , Pale or white Green, pale or white Variegated
2.	Pale or white	Green Pale or white Variegated	Green, Pale or white Green, pale or white, Variegated
3.	Variegated	Green, Pale or white Variegated	Green, Pale or white Green, pale or white Variegated

The irregularity of transmission from variegated branches could be understood by considering cytoplasmic genes (plasmagenes) of plastids. A study of the egg during oogenesis in *Mirabilis* reveals that the ooplasm contains plastids like cytoplasm of other plant cells. If the egg cell is derived from green plant tissues, its ooplasm will contain coloured plastids; if derived from white plant tissues, its ooplasm will contain white plastids; if derived from variegated tissues, its cytoplasm may contain coloured plastids only, white plastids only or a mixture of coloured and white plastids. A study of the pollenogenesis, however, reveals that pollen contains very little cytoplasm which in most cases is devoid of plastids. Without the plastids, the pollen cannot affect this aspect of the offspring's phenotype.

The pollen is devoid of plastid and cannot influence the inheritance of plastid. Thus the colour depends entirely upon the egg and what the egg produces is dependent upon the plastid of the cytoplasm. Thus it is an evidence of cytoplasmic inheritance.

In comparison to traits controlled by maternal effects, those traits controlled by maternal inheritance, the female phenotypes are always expressed in offspring. From this, it was suggested that the organelle DNA in the embryos of the four o'clock plant were inherited from the mother.

Mitotic segregation:- Variegated branches of *Mirabilis jalapa* produce three kinds of eggs. Some contain only white chloroplasts, some contain only green chloroplasts and some contain both types of chloroplasts. This process of sorting might be described as "mitotic segregation" In mitotic segregation since both segregation and recombination of organelle genotype takes place, it is called cytoplasmic segregation and recombination (its acronym is CSAR).

The results obtained from various crosses of leaf phenotypes *of Mirabilis jalapa,* as shown in Table 1 clearly indicates that leaf phenotype of the progeny is the same as that of the female parent. The phenotype of male parent did not contribute anything to the progeny.

All of the organelle DNA that is found in an embryo is from the female. The egg cell is many times larger than the pollen cells and contains both mitochondria and chloroplasts. Pollen is small and is essentially devoid of organelles, and thus organelle DNA. So any trait that is encoded by the organelle DNA will be contributed by the female.

In the case of the four o'clock plant, the different colours of the leaves are a result of the presence or absence of chlorophyll in the chloroplast, a trait that can be controlled by the chloroplast DNA. Thus, green shoots contain chloroplasts that have chlorophyll, the chloroplasts in the white shoots contain no chlorophyll and the variegated shoots contain some chloroplasts with chlorophyll and some without chlorophyll. Thus, depending on the location in the plant where the flower comes from, the egg can have chloroplast with chlorophyll, without chlorophyll, or a mixture of the two types of chloroplasts.

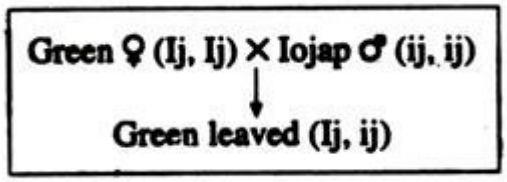
I. Inheritance of Pyrenoids in Chloroplasts of *Spirogyra triformus*

Spirogyra contains two chloroplasts in each cell. In 1920, Van Wisselingh found a cell in which one chloroplast had pyrenoids and the other chloroplast did not have pyrenoids. When this cell divided, both daughter cells were like the mother cell. Genes in the nucleus could not have caused this because both chloroplasts would have been the same. Genes in the cytoplasm outside of the chloroplast couldn't have caused this because both chloroplasts are in the same cytoplasm and would be the same. This could only be caused by genes in the chloroplast itself. This work proved that genes are present in the chloroplasts themselves, which control the phenotype of chloroplasts. Genes in the nucleus also control the phenotype of chloroplasts. There are hundreds of known genes located on chromosomes that control the phenotype of chloroplasts for instance albino gene in maize.

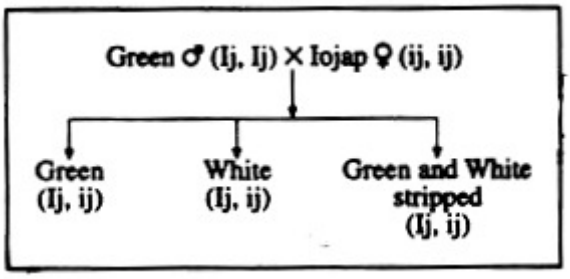
II. Maternal inheritance by *iojap* gene of maize:-

Higher plants suggest the existence of plastid genes controlling plastid integrity. A gene in corn plant called iojap (ij) has been mapped by M. Rhoades (1946) to nuclear chromosomes 7. In maize plant, iojap produces green and white stripped leaves. Plants homozygous for ij are either inviable white seedlings or variegated with a characteristic white striping, the phenotype being known as striped. The name iojap was derived from 'Iowa' state (USA), the source of maize strain and japonica, the name of a stripped variety.

When the variegated plants serve as females in a cross, they give rise to green, white, and striped progeny, regardless of the nuclear genotype of the paternal parent. Thus, if the pollen derives from a normal green Ij/Ij plant as in Fig.3, the resulting progeny will be Ij/ij heterozygotes, but many will exhibit abnormal plastid pigmentation. The presence of the "normal" Ij gene has no curative effect. When a normal plant with green leaves used as a female parent is crossed with iojap parent, the offspring will be green leaved. In the reciprocal Ij/Ij female and X ij/ij male cross produced the Ij/ij progeny which are all normally pigmented.



Again, when a reciprocal cross is made between a normal green plant (used as male) and iojap plant (used as female). The offspring will be of three different types:



In iojap plants, green and white stripped trait of leaf is inherited from the female parent due to maternal inheritance. It seems that iojap plants contain two types of plastids- normal green, and abnormal iojap plastids. During the formation of egg cells plastids are randomly distributed in the egg cells. If the egg cell receives normal green plastids it will produce green leaved plants irrespective of which plant acted as pollen parent. If the egg cell receives abnormal colourless plastids, it will give rise to white leaved plants. If the egg cell receives both green and abnormal plastids it will give rise to plants with green and white stripped leaves.

The iojap trait, thus, exhibits classical maternal inheritance once it has become established in an ij/ij plant. Moreover, once established, it becomes independent of the ij gene, as can be demonstrated by crossing F_1 Ij/ij variegated females to Ij/Ij normal males. As shown in Fig 3 a mixture of green, striped and white progeny again results, even though some of the striped and white plants now have an Ij/Ij genotype. Thus, the iojap trait, once established, is permanent.

If stripped leaved F_x iojap (I_j,i_j) as female parent is crossed with normal green leaved (Ij Ij) as male parent the following types of offspring are obtained:

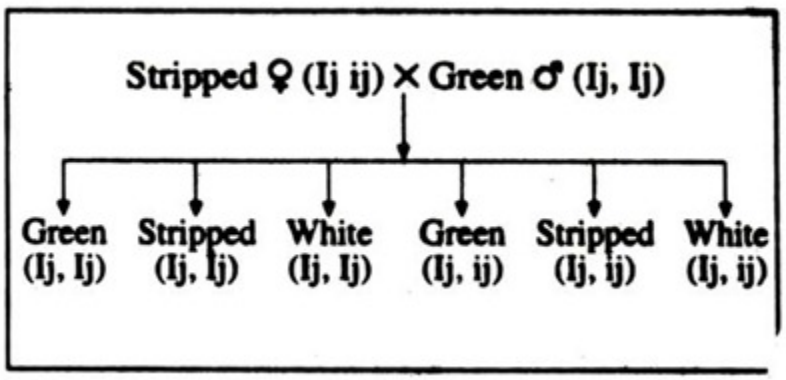


Fig 8.3: Stripped leaved F_x iojap (I_j,i_j) as female parent is crossed with normal green leaved (Ij Ij)

This backcross experiment shows that green males have no effect upon progeny. The iojap phenomenon has been explained by two hypotheses. One hypothesis holds that the ij/ij genetic constitution could bring about or permit, frequent mutations in the chloroplast genome that result the production of abnormal plastids. Another hypothesis suggests that certain cytoplasmic

elements other than chloroplast mutations come into being or residence in ij/ij cells, are later inherited in the absence of this "susceptible" or "permissive" genotype, and bring about the bleaching of chloroplasts. It is also suggested that a nuclear gene controls the development of abnormal plastids in the cytoplasm. So this type of inheritance is a case of inaction between nuclear and cytoplasmic inheritance.

8.3.1.2 Extra-nuclear inheritance by mitochondria:

The most important work on the genetics of mitochondria done in yeast was initiated by the discovery of petite mutants by B. Ephrussi (1953); subsequently mt DNA was studied in several organisms including plants and animals.

(i) Petite in yeast: Yeast (*Saccharomyces cerevisiae*) is single-celled Ascomycetes fungi. In the life cycle, diploid and haploid adult alternates, the former reproducing by asexual meiospores called ascospores, the latter by isogametes. The petite mutants in yeast fail to grow on carbon source such as glucose and produce smaller colonies (the "littles") when grown on sugars such as glucose. Since this difference can be observed only when such yeast cultures are kept in an oxygen- containing environment; it is concluded that petite mutants have a defective aerobic respiratory mechanism. In other words, slow growth of petite can be attributed to yeast cells utilization of less efficient fermentation process. These petites differ from wild type, called grande and arew characterized by (i) their insensitivity to inhibitors of aerobic pathways (such as cyanide), (ii) absence of cytochromes a, a_3, b and a number of other changes in mitochondrial respiratory enzymes, (iii) incomplete development of mitochondria and, (iv) lack of stainability of petite mitochondria.

The petite mutants can be segregational, i.e., they follow mendelian segregation and, therefore, presumably controlled by chromosomal genes. They may also be vegetative, i.e., non-segregational or extra-chromosomal. The genetic basis of petite character is a cytoplasmic factor **ρ-factor** $^{+}$ (rho) which may be absent or defective in petites. Thus, a vegetative petite can be neutral (ρ^{0}) which completely lack ρ^{+} or it may be suppressive (ρ^{-}) having a defective ρ^{+}. The neutral petites are not transmitted while suppressive petites are transmitted to a fraction of vegetative diploid progeny. In various strains of yeast, the suppressiveness varies from 1-99 per cent petites. The following two lines of evidences have suggested the association of ρ^{+} with mitochondrial DNA (mt DNA); (1) Ethidium bromide which induces petite mutations with 100 per cent efficiency, causes degradation of mt DNA after prolonged exposure of cells. In fact, neutral petites have been found lacking in mt DNA. (2) Suppressive petites base composition with respect to wild mt DNA.

(ii) Poky strain of *Neurospora*:

In fungi, *Neurospora crassa* a number of mutations of mitochondria are inherited via the female parent. The best studied of these is the poky strain of *N. crassa*, first isolated by Mitchell and Mitchell (1952). A poky mutant differs from wild type strain of *Neurospora* in the following aspects: (1) it is slow growing; (2) it shows maternal inheritance and, (3) it has abnormal cytochromes. Of the three cytochromes- Cyt a, b and c found in wild type, Cyt a and Cyt b are

absent, and Cyt c is in excess in poky mutant. In reciprocal crosses, poky character shows maternal inheritance:

Poky (female) × wild type (male) → all poky

Wild type (female) × poky (male) → all wild type

However, there are other marker nuclear genes (ad^+/ad^-) which show 1:1 Mendelian segregation. The following evidences suggested that poky trait may be located in mitochondrial DNA:

(i) Slow growth may be due to lack of ATP energy and source of this energy is mitochondria;

(ii) Cytochromes in poky strain differ from those in wild type in quality and quantity and these cytochromes are found in mitochondria.

8.4 STUDY OF MALE STERILITY IN PLANTS

Koelreuter in 1763 first reported male sterility in flowering plants. Later on numerous cases of male sterility in angiosperm were reported. A case of male sterility was discovered and carefully analysed by M. M. Rhoades (1933) in maize. In plants, the phenotype of male sterility is found to be controlled either by nuclear genes or plasmagenes (cytoplasm) or by both. He observed a male sterile plant in maize. In this plant pollens are aborted in the anther. The male sterile plant is produced when an egg cell containing cytoplasmic male sterility factor is fertilised by pollen from normal male fertile plants. It indicates that male sterility is contributed by the cytoplasm of female parent. It is also observed that when a male sterile female plant is crossed with wide range of fertile males, all progenies are male sterile in the subsequent generations. Male sterility refers to a condition in which pollen is either absent or non-functional in flowering plants. The main features of male sterility briefly presented below:

1. Male sterility is an important out breeding device which prevent autogamy and permit allogamy. In other words, male sterile plants produce seeds only on cross pollination with functional pollens of other plants.

2. Presence of male sterility leads to hetrozygosity in species as it promotes out breeding and reduce homozygosity due to elimination of inbreeding.

3. Male sterility results from action of nuclear genes or cytoplasmic genes or both. Male sterility occurs due to pollen or anther abortions.

4. Male sterility occurs in nature through spontaneous mutations as well as, can be induced artificially by chemical or physical muatgens.

5. Male sterility can be observed in all diploid species of crop plants both wild and cultivated if properly investigated. Male sterility has been reported in several crop plants (Table 2).

TABLE 2: List of some crop plants in which male sterility is found

Plant family	Name of the plant		Type of male sterility found
	English	Scientific	
Poaceae	Barley	*Hordeum vulgare*	GMS
	Wheat	*Triticum aestivum*	GMS, CGMS
	Maize	*Zea mays*	GMS, CGMS
	Sorghum	*Sorghum bicolour*	GMS, CGMS
	Pearlmillet	*Pennisetum americanum*	CGMS
	Rice	*Oryza sativa*	GMS, CGMS
	Sugarcane	*Saccharum officinarum*	CGMS
Fabaceae	Lucerne	*Medicago sativa*	GMS
Asteraceae	Sunflower	*Helianthus annuus*	GMS, CGMS
Cucurbitaceae	Watermelon	*Citrullus vulgaris*	GMS
	Muskmelon	*Cucurbita moschata*	GMS
	Pumpkin	*Cucurbita maxima*	GMS
Malvaceae	Cotton	*Gossypium hirsutum*	GMS, CGMS
Solanaceae	Tomato	*Lycopersicon esculentum*	GMS, CGMS
	Tobacco	*Nicotiana tobacum*	GMS, CGMS
Liliaceae	Onion	*Allium cepa*	CMS, CGMS
Chemopodiaceae	Sugarbeet	*Beta vulgaris*	GMS, CGMS
Linaceae	Linseed	*Linum usitatissimum*	CGMS
Apiaceae	Carrot	*Daucus carrota*	CGMS

GMS = genetic male sterility; CMS = Cytoplasmic male sterility.
CGMS = Cytoplasmic genetic male sterility.

Types of male sterility

Male sterility in plants can be controlled by nuclear genes or cytoplasm or by both. Therefore, the trait of male sterility of plants is controlled by the following three methods:

(a) Genetic male sterility (GMS):

This is the most common form of male sterility found in a number of plant species in both monocots as well as dicots. In this system the male sterility is controlled by nuclear gene and is termed as genetic male sterility. It is independent of cytoplasmic influences. In this type of male sterility, the sterility is controlled by a single nuclear gene which is recessive to fertility, so that the F_1 progeny would be fertile and in F_2 generation, the fertile and sterile individuals will be segregated in the typical 3:1 ratio (Fig 4). In most cases its expression is controlled by one or two pairs of recessive alleles, which segregate independently. This type of sterility has been reported in several crop plants like barley, cotton, cucurbits, lucerne, maize, sorghum, sugar beets, tomato and, wheat. Male sterility has some similarities and some dissimilarity with self incompatibility (Table 3).

TABLE 3: Compression on self incompatibility and male sterility

S. No.	Self incompatibility	Male sterility
Similarities		
1.	It is important out breeding mechanism.	It is also important out breeding mechanism.
2.	It prevents autogamy and promotes allogamy.	Same as self incompatibility
3.	It is found in nature.	It is also found in nature.
4.	It is used for hybrid seed production.	Also used for development of hybrids.
Dissimilarities		
5.	Pollen is functional.	Pollen is absent or non functional.
6.	It results due to morphological, genetic, physiological and biochemical causes.	It results due to genetic, cytoplasmic or both causes.
7.	Artificial induction is difficult.	Artificial induction is easy.

The mutant male-sterile plants may arise spontaneously carrying homozygous alleles (*msms*), and these will be lost if not maintained as heterozygotes (*Msms*). For this to happen, the male-sterile mutants need to be pollinated with fertile homozygous (*MsMs*) or heterozygote (*Msms*) counterparts. In cases where male sterility is controlled by dominant alleles, its maintenance through reproductive means is very difficult.

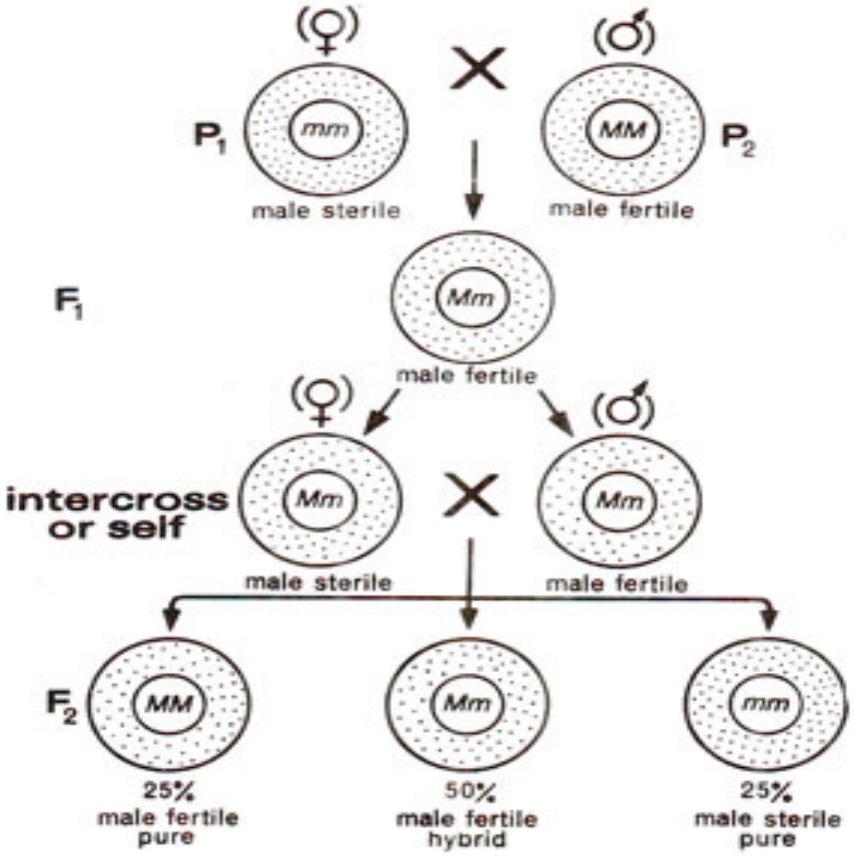


Fig 8.4: Inheritance pattern of genetic male sterility

(b) Cytoplasmic male sterility (CMS):

In this system the male sterility is controlled by cytoplasmic gene or plasmagene and, termed as cytoplasmic male sterility. It has been reported in different crop plants. Male sterile plants produce no functional pollen, but do produce viable eggs. It occurs in many plant species and is often associated with chimeric mitochondrial open reading frames. This happens due to deleterious interactions of mitochondrial genes with those present in the nucleus. The main features of cytoplasmic male sterility are given below:

1. This type of cytoplasm is designated as "sterile" (S), and it can originate spontaneously or through wide hybridization. Such plants do not produce fertile pollen grains because its nucleus also contains a pair of recessive non-restoring (*msms*) alleles.
2. CMS systems represent a valuable tool in the production of hybrid seed in self-pollinating crop species, including maize, rice, cotton, and a number of vegetable crops. Hybrid seed is produced from a cross between two genetically different lines; such seeds usually result in larger, more vigorous plants.
3. In the cytoplasmic male sterility system, the diversification of hybrid parents may be difficult due to incorporation of recessive non-restorer nuclear alleles.

4. Cytoplasmic male sterility is determined by cytoplasmic factors. Since the bulk of cytoplasm of zygote is contributed by the egg cell and the pollen tube containing male gametes contributes negligible or no cytoplasm, sterility factors present in the cytoplasm of egg cell will be transmitted to the offspring which would always be male sterile.
5. CMS can arise spontaneously in breeding lines, following mutagenesis, as a result of wide crosses, or the interspecific exchange of nuclear and cytoplasmic genomes.
6. Cytoplasmic male sterility is not influenced by environmental factors such as low or high temperature. In other words, the sterility is stable.

Origin of cytoplasmic male sterility

CMS systems have been identified and characterized in many crop species, including *Brassica napus*, *Phaseolus vulgaris*, beet, carrot, maize, onion, petunia, rice, rye, sorghum, sunflower and wheat because of their value in hybrid seed production.

In maize, three distinct male sterile sources (Cms) cytoplasm are known. These are designated as Cms-T, Cms-C and Cms-S. The normal male fertile cytoplasm is known as N- cytoplasm. Each of the three Cms cytoplasm display strict maternal inheritance even when all chromosomes are replaced from male sterile plants by a male fertile source through repeated backcrossing. The diagrammatic representation of cytoplasmic male sterility is given below in Fig.8.5:

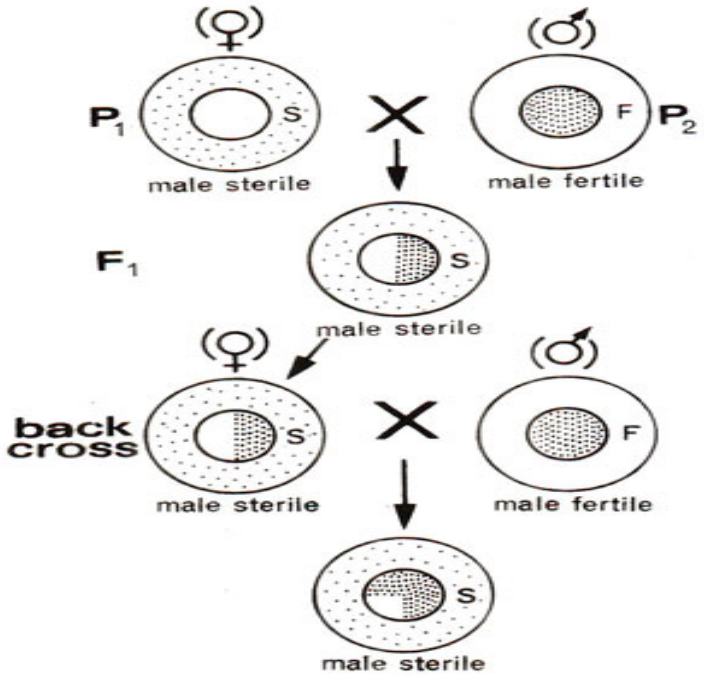


Fig 8.5: Cytoplasmic male sterility in plants

Even then male sterility characteristics could not be avoided and the characteristics will persist. It indicates that if the character is present on chromosome as nuclear gene, then male sterility could be eliminated by repeated backcrossing. Therefore, it is confirmed that male sterility is not

controlled by nuclear gene, i.e., nuclear gene has no influence on cytoplasmic male sterility. In rare cases, male sterile plants produce a few fertile pollen grains. When reciprocal crosses are made between male sterile parent (with fertile pollen) and normal male fertile (female), the progeny is found to be male fertile. Such cases confirm maternal inheritance of male sterility.

The mt DNA of S-cytoplasm contains two unique plasmid-like DNA fragments called SF (Mol. wt. 3.45×10^6) and S-S (mol.wt.4.10×10^6). These plasmid-like DNAs are not found in the isolated DNA of chloroplast or nuclei. Therefore, these plasmid-like DNAs are the characteristic of mitochondrial DNA of S-cytoplasm. This plasmid-like DNA is also absent in the mitochondrial DNA of N-cytoplasm (Normal-fertile), T-cytoplasm (male sterile) as well as C-cytoplasm (male sterile). Hence it has been suggested that such plasmid- like DNAs in mitochondria are responsible for causing male sterility. T and C-cytoplasm of male sterile plant is comparatively stable and irreversible. It means that they never give rise to fertile cytoplasm even by applying mutagens.

On the other hand, S-cytoplasm is stable. It is found to change into fertile condition in some cases due to cytoplasmic mutation from male sterile to male fertile and nuclear mutation giving rise to a new repressor gene. It is apparent that both these changes are involved to make it fertile. When a fertile line derived from S-cytoplasm is crossed as male with a Cm-S tester female plant, in some cases the offspring is male sterile. It indicates that the restorer gene is, possibly absent. In other cases the offspring is semi-fertile. It suggests that the restorer gene is possibly present. This restorer gene is different from the normal nuclear restorer gene-Rf_3 meant for S-cytoplasm.

These new restorer genes are likely to be located on different chromosomes where they are, possibly attached like episome at different time and bring the change from sterile to fertile condition. It has also been suggested that male fertility genes could be originally located on organelle DNA and were later transposed to a nuclear site giving rise to restorer gene. The gene or DNA segment that has migrated from the organelle to the nucleus or to the other organelle is termed promiscuous DNA. When this fertility gene is absent from both organelle and nucleus, this might have led to cytoplasmic male sterility. The restorer gene present in the nucleus as dominant gene generally nullifies the effect of cytoplasmic male sterility so that individuals having a restorer gene in homozygous or heterozygous state are fertile even in the presence of male sterile cytoplasm.

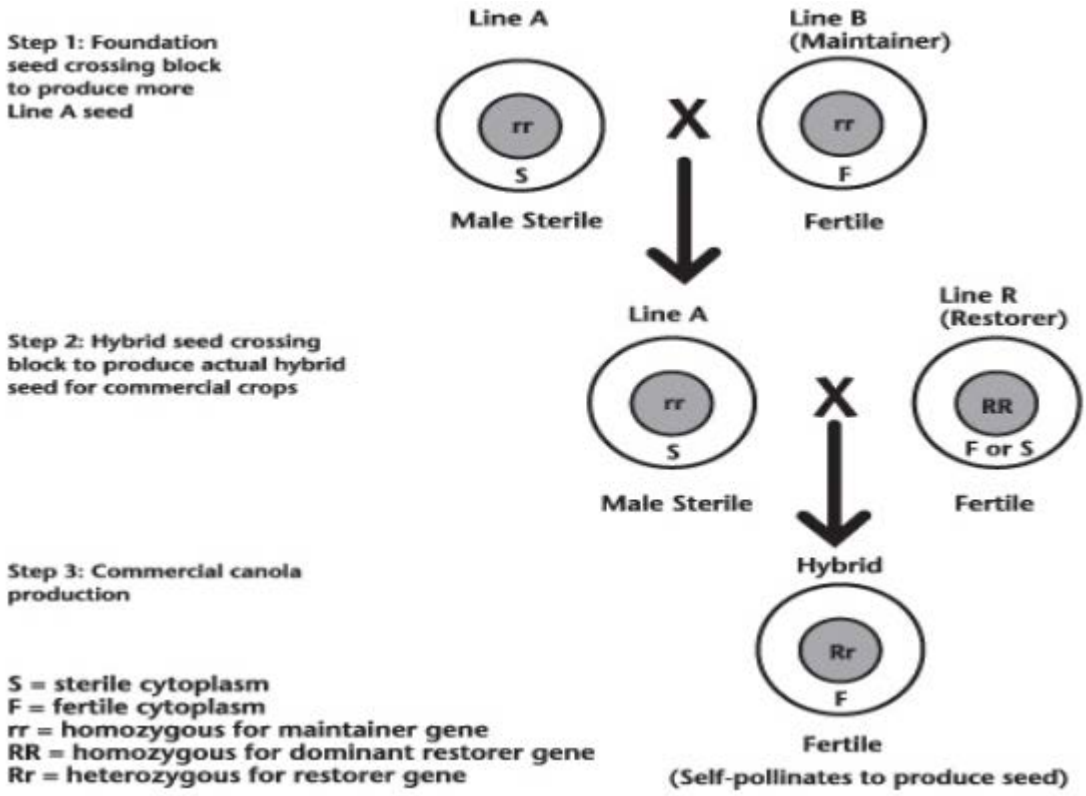


Fig 8.6: Use of cytoplasmic male sterility

In case of Cms-T, plasmid-like event in the mitochondria is absent but some unique polypeptides are produced in the mitochondria which bring the male sterility. When restorer gene is present in the nucleus, it prevents the production of unique polypeptides in Cms-T, and the plant becomes fertile. But when nuclear restorer gene is absent, the plant achieves male sterile cytoplasm. Cms-C has also two additional plasmid-like elements like Cms-S. These elements are associated with cytoplasmic male sterility.

The maternal inheritance mechanism that transmits male sterility in maize has also been demonstrated by Dhawan and Paliwal in 1964. In their experiment they used two strains of maize- Sikkim primitive-2 and another strain from Colorado-for reciprocal crosses. When Sikkim primitive-2 was used as female parent in the cross, the offspring showed little vigour and poor yield, but when Colorado strain of maize was used as female parent, the hybrid were more vigorous and showed high yield potency. These differences in hybrids of reciprocal crosses suggest that yield and vigour are governed by female cytoplasm.

(c) Cytoplasmic genetic male sterility:

When pollen sterility is controlled by both cytoplasmic and nuclear genes, it is known as cytoplasmic male sterility. This type of male sterility was first discovered by Jones and Davis (1944) in Onion. Now GCMS reported in several crops. The difference between the two types is in their fertility restoration mechanisms. In certain plants, though the male sterility is fully

controlled by the cytoplasm, but a restorer gene if present in the nucleus, will restore fertility. Further depending on the type of fertility-restoring gene, the expression of male fertility/sterility could be total or partial. Sometimes such expressions are also affected by prevailing environmental conditions such as photoperiod, temperature or both. For example, if the female parent is male sterile (due to plasmagene of male sterility) then the nuclear genotype of the male parent will determine the phenotype of F_1 progeny. Thus, if male sterile female parent contains recessive nuclear genotype rr of restorer gene and male parent is RR, having homozygous dominant restorer genes their F_1 progeny would be male fertile Rr. However, if the male parent is male fertile rr, the F_1 progeny would be male sterile rr. If the F_1 male fertile heterozygote (Rr) is test crossed with male fertile rr male, a progeny with 50 per cent male fertile and 50 per cent male sterile will be obtained. The diagrammatic representations of Inheritance of cytoplasmic genetic male sterility are given below in Fig 1.7:

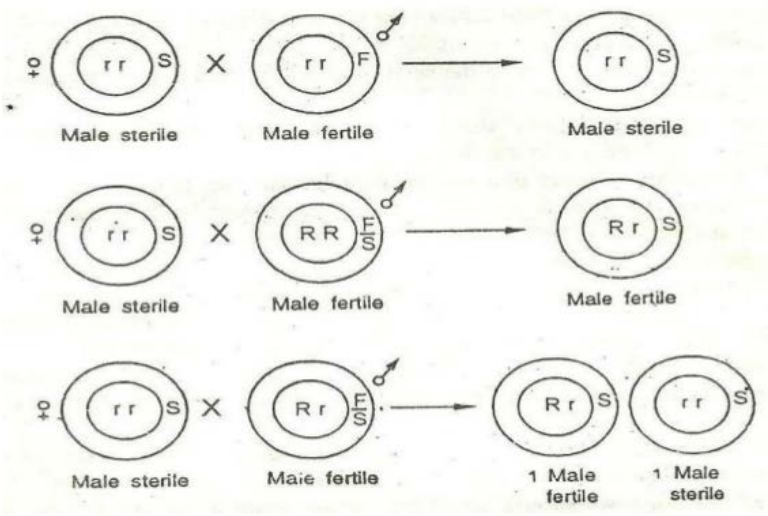


Fig.8.7: Inheritance of cytoplasmic genetic male sterility

In maize, expression of male sterility depends on an interaction between nuclear and extra chromosomal genes. Male sterile lines can bear seeds only after cross-pollination. For this reason they are useful in raising hybrid seeds, especially on large scale. Comparison of three types of male sterility is given below:

TABLE 4: Comparison of three types male sterility in crop plants

S.No.	Genetic male sterility	Cytoplasmic male sterility	Cytoplasmic genetic male sterility
1.	It is controlled by nuclear gene	It is controlled by cytoplasmic genes.	It is controlled by both nuclear and cytoplasmic genes.
2.	It consist of A & B	It consist of A & B lines	It consist of A, B & R lines

	lines		
3.	It may become fertile at low temperature.	There is no effect of temperature	There is no effect of temperature
4.	It is maintained by mating sterile plants with heterozygous male fertile plants	It is maintained by crossing of A & B lines	It is maintained by crossing of A & B lines
5.	It is used for production of hybrid, both in seeds and vegetatively propagated crops	It is used for development of hybrid in vegetatively propagated crops	It can be used in both in seeds and vegetatively propagated crops
6.	The male sterile and fertile plants are observed in 1:1 ratio. The fertile plants have to be removed from hybrid seed production	It cannot be used in seed propagated plants because the F_1 is sterile.	It requires handling of three types of material i.e. A, B & R lines
7.	Barley, cotton, musk melon, , pumpkin, rice, sunflower, tobacco, tomato, water melon wheat	Onion, sugarcane, forage crops, etc	Cotton, maize, pearl millet, rice, sorghum, sugar beet, sunflower, tobacco, tomato wheat, etc.

8.5 SUMMARY

Extra-chromosomal inheritance, extra-nuclear inheritance, somal inheritance and maternal inheritance are all synonyms. All these terms can be defined as the inheritance of characteristics of only one of the two parents, usually the female parent to the progeny. The reciprocal crosses show consistent differences as well as there is a lack of segregation in F_2 and subsequent generations. The inheritance of characters is governed by genes present in the cell cytoplasm rather than by genes on the chromosomes in the cell nucleus. The genes controlling cytoplasmic inheritance are present outside the nucleus and in the cytoplasm, they are known as plasma genes, cytoplasmic genes, cytogenes, extra nuclear genes or extra chromosomal genes. These variations suggest that the genes for the inheritance of certain characters do not occur within the nucleus, but they are present in cytoplasm and play an important role in transmission of certain specific traits, which are not controlled by nuclear genes. Therefore, it builds up the concept of cytoplasmic inheritance. The genes for cytoplasmic inheritance are independent, self-replicating nucleic acids.

Male sterility in plants is often cytoplasmically based and maternally inherited. Male sterility in plants implies an inability to produce or to release functional pollen and, is the result of failure of formation or development of functional stamens, microspores or gametes. Male sterile plants produce no functional pollen, but do produce viable eggs. Here nuclear genes do not play any significant role rather; the sterility is inherited through egg cytoplasm from generation to generation. This result indicates that chromosomal nuclear DNA does not have any significant role in male sterility (particularly in maize). Furthermore, most of the cytoplasm and organelles are inherited from the maternal side. From the scientific findings, it is confirmed that the sterility is inherited from the cytoplasm. Cytoplasmic nuclear male sterility has been extensively used by plant breeders to achieve breakthrough in the productivity of various field and horticultural crops through the development of hybrid cultivars. The impact of this technology is visible in crops like maize, pearl millet, rice, sorghum, etc., and this has helped in encountering the challenges of global food security.

8.6 GLOSSARY

A line: The male sterile line.

B Line: Isogenic line of A line with male fertility.

Carrier: A healthy person possessing a mutant gene in heterozygous form: also refers to a person with a balanced chromosomal translocation.

Chromosome: A molecular "package" for carrying DNA in cells, organized as two double-helical DNA molecules that encode many genes. Some simple organisms have only one chromosome made of circular DNA, while most eukaryotes have multiple chromosomes made of linear DNA. Different organisms have different numbers of chromosomes.

Cytoplasm: It is the gelatinous liquid that fills the inside of a cell. It is composed of water, salts, and various organic molecules. Some intracellular organelles, such as the nucleus and mitochondria, are enclosed by membranes that separate them from the cytoplasm.

Cytoplasmic genetic male sterility: Pollen sterility that is controlled by both cytoplasmic and nuclear genes.

Cytoplasmic inheritance: Inheritance of traits through DNA that is not connected with the chromosomes but rather to DNA from organelles in the cell.

Cytoplasmic male sterility: Pollen sterility caused by cytoplasmic gene.

F_1: Its full name is first offspring (filial generation). It is the first generation obtained by hybridization. The next and subsequent generations are referred to as F_2, F_3, etc.

F_2: It is the second generation obtained on self fertilization of F_l.

F_3: It is the third generation obtained on selfing of F_2 hybrids.

Gamete: Cells formed after meiosis is called gametes. Gametes are haploid (reproductive) cells. Female gametes are called ova or egg cells and male gametes are called sperms.

Genes: Units of inheritance usually occurring at specific locations or loci on a chromosome. Physically, a gene is a sequence of DNA bases that specify the order of amino acids in an entire

protein or, in some cases, a portion of a protein. A gene may be made up of hundreds of thousands of DNA bases. Genes are responsible for the hereditary traits in plants and animals.

Genetic male sterility: Pollen sterility caused by nuclear gene.

Genome: The total genetic information of an organism, cell, or species.

Genotype: The genetic makeup of an organism determined by the particular combination of alleles at one or more specific locations (loci) on one or more paired chromosomes.

Heterozygous: Carrying two different alleles for a particular gene.

Homologous chromosomes: Chromosomes that are paired during the production of sex cells in meiosis. Such chromosomes are alike with regard to size and also position of the centromere. They also have the same genes, but not necessarily the same alleles, at the same locus or location.

Homozygous: Having the same allele at the same locus on both members of a pair of homologous chromosomes. Homozygous also refers to a genotype consisting of two identical alleles of a gene for a particular trait. An individual may be homozygous dominant (AA) or homozygous recessive (aa).

Hybrid: Offspring that is the result of mating between two genetically different kinds of parents.

Isogenic Lines: Line having single gene differences.

Male sterility: A condition in which either pollen is absent or non-functional in flowering plants.

Meiosis : The stage in which sperm and egg cells are formed. It is during this process that the autosomal chromosomes recombine and mutations occur.

Mendelian inheritance: It refers to patterns of inheritance that are characteristic of organisms that reproduce sexually. The Austrian monk Gregor Mendel performed thousands of crosses with garden peas at his monastery during the middle of the 19th century. Mendel explained his results by describing two laws of inheritance that introduced the idea of dominant and recessive genes.

Mitochondria: Membrane-enclosed structures within cells that generate most of the cells energy through the production of adenosine triphosphate (ATP), a molecule that provides the energy needed for many key metabolic reactions

Mitochondrial DNA: It is the small circular chromosome found inside mitochondria. The mitochondria are organelles found in cells that are the sites of energy production. The mitochondria, and thus mitochondrial DNA, are passed from mother to offspring.

Mutation: A mutation is a change in a DNA sequence. Mutations can result from DNA copying mistakes made during cell division, exposure to ionizing radiation, exposure to chemicals called mutagens, or infection by viruses. Germ line mutations occur in the eggs and sperm and can be passed on to offspring, while somatic mutations occur in body cells and are not passed on.

Nucleus: A nucleus is a membrane-bound organelle that contains the cell's chromosomes. Pores in the nuclear membrane allow for the passage of molecules in and out of the nucleus.

Organelle: An organelle is a sub cellular structure that has one or more specific jobs to perform in the cell, much like an organ does in the body. Among the more important cell organelles are

the nuclei, which store genetic information; mitochondria, which produce chemical energy; and ribosome, which assemble proteins.

Ovum (plural ova): A female (reproductive) sex cell or gamete.

Phenotype: A phenotype is an individual's observable traits, such as height, eye color and blood type. The genetic contribution to the phenotype is called the genotype. Some traits are largely determined by the genotype, while other traits are largely regulated by environmental factors.

Plasmid: A plasmid is a small, often circular DNA molecule found in bacteria and other cells.

R line: Line that restore fertility when crossed with cytoplasmic genic male sterile lines.

Recessive: An allele that produces its characteristic phenotype only when the paired allele is the same; will be masked if a dominant allele is present. Trait expressed in people who are homozygous or hemizygous for a particular gene, but not in those who are heterozygous for the gene.

Trait: A trait is a specific characteristic of an organism. Traits can be determined by genes or the environment, or more commonly by interactions between them. The genetic contribution to a trait is called the genotype. The outward expression of the genotype is called the phenotype.

Zygote: A "fertilized" ovum. More precisely, this is a cell that is formed when a sperm and an ovum combine their chromosomes at conception. A zygote contains the full complement of chromosomes (in human 46) and has the potential of developing into an entire organism.

8.7 SELF ASSESSMENT QUESTIONS

8.7.1 Multiple choice questions

1. The chromosomal theory of inheritance was proposed by

(a) Mendel (b) Watson and Crick

(c) Darwin (d) Sutton and Boveri

2. Which of the following doesn't agree with the chromosomal theory of inheritance?

(a) The genes are located on the chromosomes

(b) The genes on the same chromosome are always pass together

(c) The genes are located linearly on the chromosomes

(d) The distance between genes can be mapped

3. Transmission of genes that occur outside nucleus is called

(a) Extranuclear inheritance (b) Cytoplasmic inheritance

(c) Both A and B (d) None of above

4. Extranuclear inheritance commonly occurs in

(a) Nucleus (b) Cytoplasmic organelle

(c) Ribosome (d) Cell membrane

5. Cytoplasmic male sterility in maize is manifestation of interactions between

(a) Chloroplast and nuclear genes (b) Mitochondrial and nuclear gene

(c) Chloroplast and mitochondrial genes (d) Cytoplasmic factors and male sterile genes

6. The two organelles responsible for cytoplasmic inheritance among eukaryotes are
(a) Lysosomes and Mitochondria (b) Mitochondria and Golgi complex
(c) Chloroplast and mitochondria (d) Chloroplast and lysosomes

7. After crossing two plants, the progenies are found to be male sterile. The phenomenon is found to be maternally inherited and is due to some genes which reside in
(a) Nucleus (b) Chloroplast
(c) Mitochondria (d) Cytoplasm

8. Extranuclear inheritance is a consequence of presence of genes in
(a) Ribosomes and chloroplasts (b) Lysosomes and ribosomes
(c) Mitochondria and chloroplasts (d) Endoplasmic reticulum and mitochondria

9. Genes for cytoplasmic male sterility in plants are generally located in
(a) Mitochondrial genome (b) Cytosol
(c) Chloroplast genome (d) Nuclear genome

10. Heterozygous individuals that can pass on recessive, abnormal conditions even if not expressing the disease are
(a) Carriers (b) Phenotypically challenged
(c) Recessively compromised (d) Zygotic

11. The hybrid produced by cytoplasmic fusion is called
(a) Sexual hybrid (b) Parasexual hybrid
(c) Somatic hybrid (d) Protoplastic hybrid

12. Cytoplasmic inheritance can be studied by:
(a) Test cross (b) Back cross
(c) Reciprocal cross (d) None
13. In zygote maximum part is coming from
(a) Male parent (b) Female parent
(c) New synthesis (d) None

14. In four o'clock plant normal leaves A and veriegated leaves B occur in different plants, if B male is crossed with A female the hybrid has normal leaves but when B female is crossed with A male the hybrid has veriegated leaves , it is a case of
(a) Mutation (b) Cytoplasmic inheritance
(c) Complementary genes (d) Supplementary genes

8.7.1 Answer Key:

1. (d), **2.** (b), **3.** (c),**4.** (b), **5.** (d), **6.** (c), **7.** (d), **8.** (c), **9.** (a), **10.** (a), **11.** (c), **12.** (c), **13.** (c), **14.** (b)

8.7.2 Very Short Answer Type Questions

a. Plasmagenes
b. Maternal inheritance
c. Male sterility
d. Petite in yeast
e. Cytoplasmic genetic male sterility
f. Breakdown of male sterility

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8.10 TERMINAL QUESTIONS

8.10.1 Long Answer Type Questions

1. Distinguish between cytoplasmic inheritances and nuclear inheritance.
2. What are the specific properties of chromosomal genes?
3. Distinguish between self incompatibility and male sterility.
4. Distinguish between genetic and cytoplasmic male sterility.
5. Discuss the role of chloroplasts and mitochondria in the cytoplasmic inheritance.
6. Given seed from a male sterile line of corn, how would you determine if the sterility was genetic or cytoplasmic?
7. Which parent contributes more in cytoplasmic inheritance?
8. Why are most organelle genomes transmitted maternally?
9. Discuss about Mitochondrial and chloroplast DNA. Why its inheritance does not follow Mendelian patterns?

10. What are the risks involved in the use of single source of male sterility systems in crop plants?
11. What kind of male sterility can be used in crops where seed is not important commercial products?
12. What is male sterility? What are different types of male sterility found in crop plants?
13. How would you differentiate between genetic and cytoplasmic male sterility? How would you induce male sterility in crop plants so as to exploit heterosis?
14. Define male sterility. List various type of male sterility found in nature and how these have been utilized in the exploitation of hybrid vigour and population improvement program of both sexually and vegetatively propagated plants.
15. Describe genetic, cytoplasmic and cytoplasmic genetic male sterility system in crop plants. Explain their utility and cite suitable examples.
16. A male sterile plant was found in a variety of maize. It was used as female and crossed with male fertile plants. The F_1 of cross was found to be fertile. What kind of male sterility this could be and why?
17. What is genetic male sterility? Give its main features and method of maintenance.
18. Define cytoplasmic male sterility? Give its main features and method of maintenance and use in plant breeding.
19. Discuss briefly various source of male sterility in crop plants.
20. A four o'clock plant with three kinds of branches (green, variegated and white) is used in a breeding experiment. What kinds of progeny are to be expected from each of these crosses? (a) Green female X white male (b) white female X green male? (c) Variegated female X green male?

UNIT-9 SEX DETERMINATION AND SEX LINKED INHERITANCE

9.1 OBJECTIVES

After reading this unit students will be able-

- To study discovery and structure of sex chromosomes
- To understand the mechanism of sex determination
- To study sex linked inheritance

9.2 INTRODUCTION

A sex-determination system may be a biological system that determines the development of sexual characteristics in an organism whether it is male, female and hermaphrodite. Most organisms that create their offspring using sexual reproduction have two sex i.e. male sex and female sex. However, some of lowest forms of plants and animal life have several sexes. For an example a ciliated protozoa *Paramecium bursaria* exhibited eight sex or mating types, all morphological identical. Occasionally, there are hermaphrodites in place of one or both sex. There are also some species that are only one sex because of parthenogenesis, which is a reproductive strategy that involves development and growth of female without fertilization.

The sex cells and reproductive organs form primary sexual characteristics of male and female sex. Besides these primary sexual characteristics, the male and female sex differs from each other in many somatic characters referred to as secondary sexual characters.

In several species, sex is determined genetically i.e. male and female organisms have completely different alleles or even different genes that specify their sexual morphology. In animals this can be accompanied by chromosomal differences, generally through combinations of XY, ZW, XO, ZO chromosomes, or haplodiploidy. The sexual differentiation is generally triggered by a main gene, a "sex locus", with a mess of other genes following in a domino effect.

9.3 STUDY OF DISCOVERY AND STRUCTURE OF SEX CHROMOSOMES

Discovery of Sex chromosomes

In a vast majority of animals, male and female individuals ordinarily differ from each other in respect of either the number or the morphology of the homologues of one chromosome pair, this pair is referred to as sex chromosome or allosome. On the other hand, those chromosomes whose number and morphology do not differ between males and females of a species referred to as autosomes. The chromosomes are responsible for the determination of sex are known as sex chromosomes e.g., X and Y chromosomes whereas the chromosomes have no relation with the sex and contain the genes which determine the somatic characters of the individuals are known as autosomes.

Structure of sex chromosomes

There are two types of sex chromosomes i.e. X and Y which exhibit structural differences. Individuals having two X chromosomes (XX) are female; individuals having one X chromosome and one Y chromosome (XY) are male. The cytogenetic studies exhibited that X chromosomes of most organisms are straight, rod like and comparatively larger than Y chromosomes. The Y chromosomes are smaller in size with one end slightly curved to one side in *Drosophila*; in man no such curvature of Y chromosomes occurs (Fig 1). The X- chromosome is found in both males and females, although one sex has only one X chromosomes while the other sex has two X chromosomes. In contrast, the Y-chromosome ordinarily occurs only in one of the two sexes of a species, e.g. male mice, *Drospohila*, human, etc., and female birds, reptiles, etc.

The X-chromosomes have large amount of euchromatin and small amount of heterochromatin. The euchromatin has large amount of DNA materials, hence, encode much genetic information of cell. The Y chromosome contains small amounts of euchromatin and large amount of heterochromatin, thus having little genetic information; therefore, sometimes it is referred to as genetically inert or inactive.

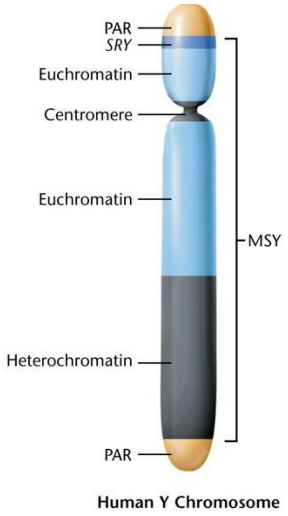


Fig.9.1: Structure of human Y chromosome

PAR stands for Pseudo autosomal region and SRY is the gene triggering male development.

9.4 STUDY OF MECHANISM OF SEX DETERMINATION

9.4.1 Sex Determination in Dioecious Organisms:

Sex determination is important to distinguish an organism as male, female and hermaphrodite. The dioecious animals have distinct male and female individuals. In dioecious diploidic organisms following two systems of sex chromosomal determination of sex have been recognized:

(a) Heterogametic males

(b) Heterogametic females

(a) Male Heterogamety: In this type of sex chromosomal determination of sex, the female sex has two X-chromosomes (XX), while the male sex has only one X chromosome. In some cases, male may possess Y chromosomes. Because, male lacks a X chromosome, therefore, at the time of gametogenesis it produces two types of gametes. 50 per cent gametes carry the X-chromosomes, while other 50% without 'X' chromosome. Such as male sexes which produce two different types of gametes in terms of sex chromosomes is called heterogametic sex. While the female sex produce similar type of gametes therefore called homogametic sex.

Types: The heterogametic males are of two types.

(i) XX-XO types: In some insects especially those of the orders Hemiptera (true bugs) and Orthoptera (grasshoppers and cockroaches) and certain plants (e.g., *Vallisneria spiralis*, *Dioscorea sinuata*, etc.), the females are homogametic XX type, while the male have only one X chromosome (hence referred to as XO). There is no 'Y' chromosome. Hence the chromosome number of the male and female will be different. The male has chromosome number one less than that of female.

The presence of an unpaired X chromosome determines the male sex. The male lacking in one X chromosome produces two types of sperms: half of the sperms have one X chromosome; while the other half have none. The union of a sperm having a X chromosome with an egg produces a zygote having two X chromosomes (XX); such zygotes develop into female individuals. But when a sperm without an X chromosome fertilizes an egg, an XO zygote is obtained which develop into males. Thus, one half of the progeny from each mating are female, while the other halves are males. The sex of offspring depends upon the sperm that fertilizes the egg (Fig 2.).

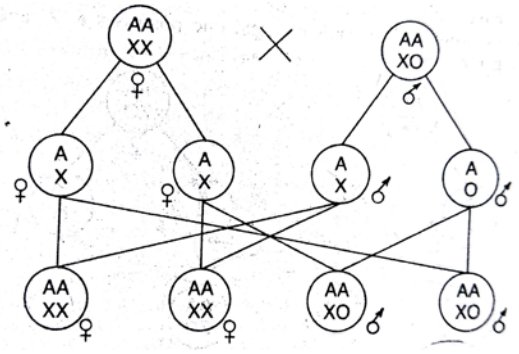


Fig.9.2: A cross showing male heterogamy (XX-XO type)

(ii) XX-XY type: In humans, mice and most of the other mammals, certain insect including Hemiptera, Coleoptera, Diptera (e.g., *Drosophila,* house fly, etc.) some fishes, some amphibian, some snakes, and in certain angiospermic plants such as *Coccinia indica*, *Humus lupulus*, *Silene latifolia*, the females possess two homomorphic X chromosomes in their body cells ((hence, referred to as XX) and they being homogametic, produce one kind of eggs, each with one X chromosome.

The males of these organisms possess one X chromosome and one Y chromosome (XY). The males having two heteromorphic sex chromosomes produce two kinds of sperms: half with X chromosome and half with Y chromosome. Fertilization of an egg by a sperm having an X chromosome yields an XX zygote, which develops into a female. But zygotes produced by the union of an egg with a sperm having a Y chromosome produces an XY zygote which produce males. A cross showing the male heterogamy is presented in Fig.9.3.

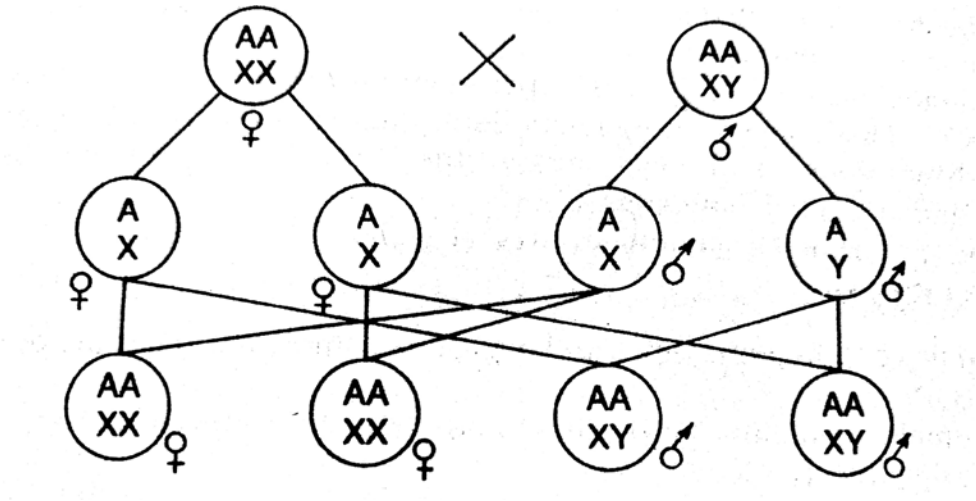


Fig.9.3: A cross showing the male heterogamy (XX-XY type)

(b) Females Heterogamety: In birds, moths, and some fish, the females are heterogametic while males are homogametic. In this type of sex chromosomal determination of sex, the male sex possesses two homomorphic Z chromosomes, therefore, is homogametic and produces single type of gametes, each carries a single Z chromosome.

The female sex either consists of single Z chromosome or one Z chromosome and one W chromosome. The female sex is thus heterogametic and produces two types of eggs, half with a Z chromosome and half without a Z chromosome (with or without a W chromosome). To avoid confusion with that of XX-XO and XX-XY types of sex determining mechanisms, instead of the X and Y alphabets, Z and W alphabets are generally used respectively. They are represented as ZW for females and ZZ for males, and the mechanism, as ZZ-ZW system. The heterogametic females showed 2 types in ZZ-ZW system:

1. ZZ-ZO type

2. ZZ-ZW type

(i) ZZ-ZO system: This system of sex determination is known in a few insect species, e.g., Fumea (*Psyche crassiorella*, a moth of Paychidae family). In moth, butterflies, and domestic chickens; females are the heterogametic (producing two kinds of eggs, half with a Z chromosome and half without any Z chromosome) and males are the homogametic sex (producing single type of sperms, each of which carries a single Z chromosome.

The union of a sperm with Z chromosome containing egg produces ZZ zygote which develops into males. But fertilization of an egg devoid of a Z chromosome with a sperm gives rise to a ZO zygote which develops into females. The sex of the offspring depends on the kind of egg shown below in Fig. 9.4.

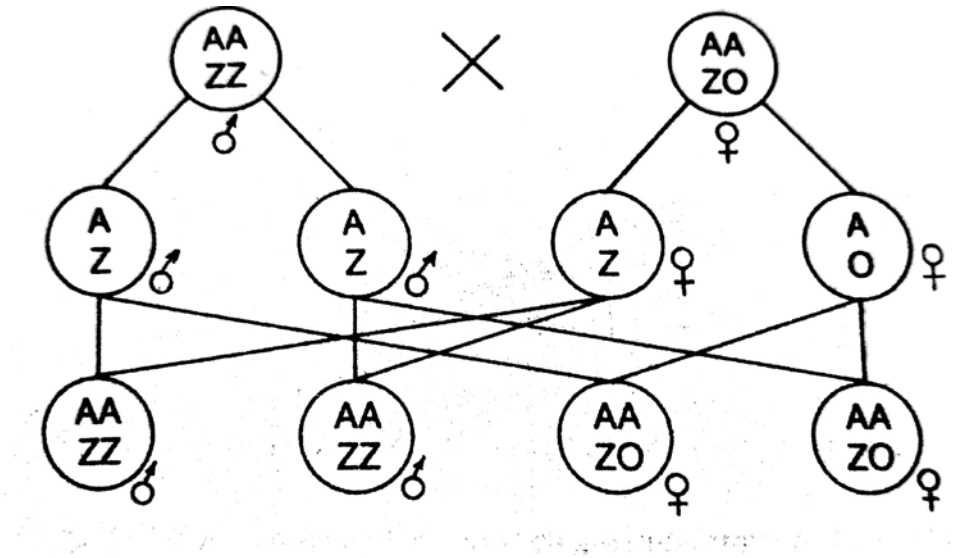


Fig.9.4: A cross showing the female heterogamy (ZO-ZZ type)

(ii) ZZ-ZW system: This system of sex determination operates in vertebrates such as fishes, birds and reptiles, some insects, e.g., silk worm, and plants such as *Frgaris elatior*, etc. Here the females have ZW chromosome constitution therefore it is the heterogametic sex as half the eggs have Z, while the rest have a W chromosome.

The male of these species have two Z chromosomes (ZZ); as a result, male is the homogametic sex since all the sperms produced by males, have one Z chromosome. Fertilization of a Z containing egg with a sperm gives rise to a ZZ zygote, which develops into a male. A ZW zygote is produced when a W containing egg is fertilized by a sperm, such a zygote develops into a female.

Significance

1. Z chromosome is bigger similar to X chromosome and carries more genes.
2. Gene sharing is not seen between avian ZW and mammalian XY system.
3. The determination of sex of the offspring depends on the ovum in contrast to the sperm in XY system.

9.4.2 Sex determination by Genetic Balance:

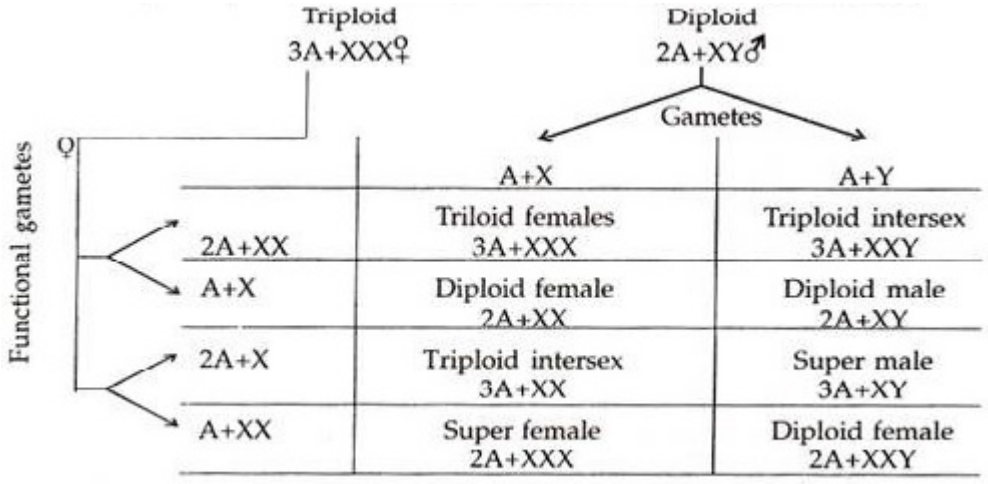
In *Drosophila melanogaster*, mice, man, etc., the presence of XX and XY chromosome is commonly associated with femaleness and maleness respectively. It seems as if the specific (X and Y) chromosomes themselves determine the sex of zygotes.

However, intense studies on gene action showed that some specific genes located in these chromosomes should be involved in sex determination. A conclusion to this effect was reached by Calvin Bridges (1926) who finally proposed his well recognised genic balance theory of sex determination in *Drosophila melanogaster*.

In 1926, Bridges discovered triploid intersexes XXY females and XO males in *Drosophila* where as finding out the inheritance of vermillion eye gene located within the X chromosome. This study clearly showed that XX and XY chromosome constitutions were not essential for femaleness and maleness respectively and that, Y chromosome did not play a role in sex determination.

A little later, in *Drosophila,* Bridges observed certain female flies which have 3 sets of autosomes and 3X chromosomes (triploid). These females when mated with normal diploid males gave rise to a progeny having number of aneuploid situations. Bridges developed the genic balance theory of sex determination by correlating the sex of an individual with its chromosome constitution (Table-1). This theory totally explains the sex determination mechanism in *Drosophila*, and possibly is applicable to birds as well.

TABLE-1: Bridges's cross of triploid (3A+XXX) female fly and a diploid (2A+XX) male fly (*Drosophila*)



The triploid female produces four kinds of eggs:

a. Egg containing two autosome sets and 2X (AAXX)
b. Egg containing two autosome sets and one X (AAX)
c. Egg containing one autosome sets and two X (AXX)
d. Egg containing one autosome sets and one X (AX)
e. Egg containing two autosome sets and one X (AAX)

The diploid male on other hand produces only two types of gametes; one containing AX and other having AY. The fertilization of the four kinds of eggs with two kinds of sperms would

result in the production of the following eight kinds of zygotes. These zygotes develop in to distinct types of flies. These are follows:

1. Individual are triploid females i.e. having three Autosomes set and three Xs (AAAXXX)
2. Flies are normal diploid (AAXX)
3. Flies are neither males nor females but intersexes (3As and 2Xs) i.e., AAAXXX
4. Individual, are super female with 2As and 3X
5. Flies are also intersexes with 3A and 2X
6. Individuals are normal males with 2A,1X and 1Y
7. Flies are also super females with 3A,1X and 1Y
8. Some flies are normal diploid females with 2A,2X and 1Y

Bridge reported that in *Drosophila* the femaleness is not simply determined by presence of two X and maleness by the presence of one X and one Y because cross gave rise to certain flies which had two X but these were not female. So there were flies, which had a Y chromosome, were still females.

The theory of genic balance by Bridge revealed that the sex of an individual is determined by a balance between the genes for maleness and those for femaleness present in the individual. In *Drosophila,* genes for maleness are present in autosomes, while those for femaleness are located in the X chromosome. He, therefore, concluded that the sex in this insect is determined by the interaction of genes on autosome with those on the X chromosomes. According to him, the autosomes carry genes for maleness and Xs for femaleness.

The genes for femaleness associated with a single X chromosome are stronger than those for maleness present in one set of autosomes, i.e. the haploid set of autosomes. The genes strength for maleness and femaleness is so balanced that when the number of X chromosomes and that of autosomal sets in equal in an individual, it develops into female.

An individual develops into a male only when the number of its X chromosomes is exactly half of the number of its autosomal sets. The sex of an individual is determined by the ratio of the number of its X chromosomes and that of its autosomal sets, this ratio is termed as sex index and is expressed as follows:

Sex index = Number of X chromosomes (X)/ Number of autosomal sets (A) = X/A

Sexual phenotype of *Drosophila melanogaster* is determined by the ratio of the number of X chromosomes to the number of sets of autosomal chromosomes (X/A ratio). As shown in the **Table 2,** when the X/A ratio is 1.0, the individual will be female and if it is 0.50, it would be male. When this balance is disturbed, the sex of individuals deviates from normal male or normal female. For example, when the X/A ratio falls between 1.0 and 0.50, it would be intersex; *(individuals with a mixture of male and female characteristics)* when it is below 0.50, it would be supermale (super males are sterile and weak) and when above 1.0, it would be super female *(*super females have severe developmental problems, and they usually do not emerge from pupal

stage). These observations also reveal that, in *Drosophila*, the Y chromosome do not play any role in determining sex of individuals.

TABLE 2: Sex expression in *Drosophila* as a function of the ratio of the number of x chromosomes and the number of autosomal sets present in an individual

Phenotypes	Number of X chromosomes = X	Number of autosome sets = A	Set index (X/A)
Super female	3	2	3/2=1.5
Tetraploid	4	4	4/4=1.0
Triploid	3	3	3/3=1.0
Diploid	2	2	2/2=1.0
Haploid	1	1	1/1=1.0
Intersex	2	3	2/3=0.67
Normal male	1	2	1/2=0.50
Super male	1	3	1/3=0.33

Gynandromorphs: Concepts of sex determination for *Drosophila melanogaster* demonstrated the occasional occurrence of gynandromorphy i.e., individuals during which one part of the body expressed male and the other part expressed female features. The male phenotype in gynandromorphs may extend to about one half of the body. In some flies, male and female parts run longitudinally, whereas in some others they run transversely. Gyanandomorphs represent one kind of mosaic or an organism made up of tissues of male and female genotype. Gynandromorphs are always mosaics for the X chromosome; the parts with male phenotype are always XO, while those with the female phenotype are XX. For example, a bilateral gynandomorph of *Drosophila* is male on one side and female on the other. Gynandromorphs arise from XX zygotes. During embryonic development in one or more cells one of the two chromosomes does not pass to any pole. Therefore, one or more daughter cells are produced with a single X chromosome. These cells divide and separately give rise to the male parts of gynandromorphs.

If the irregular distribution of X chromosome occurred at the first mitotic division of a zygote, the male phenotype would extend to exactly half the body of the gynandromorph. The extent of male part in a gynandromorphy depend on the stage of embryonic development; the earlier the occurrence, the large the size of male portion. The cytological examination of gynandomorphs suggested that a Y chromosome does not play any role in the determination of sex in *Drosophila.*

Sex Determination in Y-linked Genes

In mammals, amphibians e.g. Axolotl and some plants, e.g. *Melandrium*, *Dosophila* like XX-XY type sex determining mechanism occurs but here the Y chromosomes contain potential male sex determining genes which is essential for the development of maleness and which can almost completely overcome the minimizing action of the rest of the genotypes. The conclusive

evidence that Y chromosomes is a determiner of fertility and sex of male individual came from certain abnormal conditions which contain aneuploid sex chromosomal abnormalities.

In human, XO, XX, XXX and XXXX individuals develop the female phenotype. But in the presence of a single Y chromosome, these individuals, i.e. XY, XXY, XXXY and XXXXY, develop the male phenotype. Thus a single Y chromosome is sufficient to overcome the effects of four X chromosomes and to produce a male phenotype. But normal human females and males are produced only by XX and XY chromosome constitutions, respectively.

The XO condition produces Turner's syndrome (sterile female) having certain abnormalities such as sort stature, congenital malformation, shield chest, pronounce webbing of neck, short fourth metacarpal, colour blindness, etc., while XXY lead to the development of Klinefelter's syndrome (sterile male) despite the presence of two X chromosomes. The male determining capacity appears to be located in the short arm of human Y chromosome (Y^s); a deletion of Y^s permits the development of normal female phenotype even in XY individuals. A person with extra one X and Y chromosome display true hermaphroditism having both ovarian and testicular tissues and variable degree of intersexual development of genetalia.

1. Male Haploidy or Haplodiploidy Mechanism

Male haploidy or haplodiploidy or arrhenotokous parthenogenesis is particularly common in the hymenopterous insects such as ants, bees and wasps (e.g., *Bracon hebetor*). In these insects, fertilized eggs develop into diploid females and unfertilized ones into haploid males; so arrhenotoky is both a form of reproduction and a means of sex determination.

During spermatogenesis, all the N chromosomes of males regularly pass to a single pole at anaphase-I so that the opposite pole receives no chromosome at all. Thus all the sperms are regularly haploid. Normal meiosis during oogenesis produces all haploid eggs. Fertilization of eggs produces diploid zygotes which develop into diploid larvae. Ordinarily, such larvae give rise to workers, which are sterile females. But the diploid larvae fed on royal jelly develop into fertile females called queen. On the other hand, unfertilized eggs develop parthenogenitically to produce haploid larvae and ultimately fully fertile haploid males called drones.

2. Single Gene Control of Sex

In many animals, single autosomal genes override the effect of sex chromosomes present in the individuals. These genes are generally recessive, but in some cases may be dominant. A classical example of such a gene is that the autosomal recessive transfer (tra) gene of *Drosophila*. When this gene is available in homozygous state (tra/tra) it transforms XX zygotes to develop into males which are sterile. The gene tra has no effect either in the males or when it is in the heterozygous state in females.

When a female heterozygous for tra (XX Tra/ tra) is mated with a male homozygous for tra (XY tra /tra), only 25% of the progeny are females, while the remaining 75% are males. One-third of the males, however are sterile XX individuals homozygous for tra. They are transformed to maleness by the gene tra. A similar recessive, possibly autosomal, testicular feminization gene

induces XY human to develop breasts and vagina. Such individuals have degenerate testes and are sterile. This gene does not affect the characteristics of female individuals.

9.5 STUDY OF SEX LINKED INHERITANCE

In an XY chromosomal system of sex determination, the pattern of inheritance for loci on the heteromorphic sex chromosomes differs from the pattern for loci on the homomorphic autosomal chromosomes because alleles of the sex chromosome are inherited in association with the sex of the offspring. Alleles on a male's X chromosome go to his daughters but not to his sons, because the presence of his X chromosome normally determines that his offspring is a daughter. The inheritance of hemophilia (failure of blood to clot), is due to an allele located on the X chromosome and, is known since the end of the eighteenth century. It was also known that mostly men had the disease, whereas women could pass on the disease without actually having it. Thomas Hunt Morgan, a famous geneticist, very clearly demonstrated that a specific trait namely, white eye color, was linked to the sex chromosome. Since the Y chromosome in males did not possess any gene for eye color it gave a distorted segregation in the progeny. Therefore, a white-eyed female will produce white eyed male progenies, but in the female progenies one may expect 50% white-eyed and 50% red-eyed individuals. Now it is very well known that the distorted segregation is because genes of eye color and sex determination are tightly linked on the X chromosome. This was the first instance to prove the existence of linked genes.

Sex determination in human results from the action of a pair of chromosomes, the **sex chromosomes** (the other 22 pairs are called autosomes). Those chromosomes, which are responsible for the determination of the sex of an organism, are termed as sex chromosomes or X chromosomes. All other chromosomes of the cell are **autosomes**. There are two types of sex chromosomes: X chromosomes and Y chromosomes. X chromosomes in homozygous conditions determine femaleness and association of X chromosomes and Y chromosomes produce maleness. An understanding of Mendelian principles allows us to predict that males and females are likely to be conceived with equal probability.

The X chromosome contains a significant number of genes. In contrast, the Y chromosome contains very few. Therefore, genes on the X chromosome (often called 'X-linked' genes) are in a unique situation. Females have two copies of each gene, just like the normal situation, with autosomal (in human, there are 22 pairs of autosomes) genes. Males, on the other hand, since they have only one X chromosome, have only one copy of each X-linked gene. Because of this, males cannot be homozygous or heterozygous; they are referred to as hemizygous (The condition of having only one allele of a pair.) Therefore, alleles that are recessive in a female are automatically expressed in a male (because there is no second allele to overshadow the recessive one). The normal rules of dominance do not apply to males in this case. For this reason, problems associated with X-linked recessive alleles, such as hemophilia (inability of blood to clot) and color blindness is more common in males than they are in females.

Before we continue, we need to make a small distinction. Since both X and Y are sex chromosomes, three different patterns of inheritance are possible, all sex linked (for loci found only on the X chromosome, only on the Y chromosome, or on both). However, the term **sex linked** usually refers to loci found only on the X chromosome. The term **Y-linked** is used to refer to loci found only on the Y chromosome, which control **holandric traits** (traits found only in males). Loci found on both the X and Y chromosomes are called *pseudoautosomal.* In human beings, at least four hundred loci are known to be on the X chromosome; only a few are known to be on the Y chromosome.

In mendelian experiments it is immaterial, whether a particular character bearing parent is male or female i.e.; reciprocal crosses give same result. However, in dioecious individuals there can be two types of characters, one which do not show any different on reciprocal crosses and the other which show difference in reciprocal crosses. Former type of characters is located on autosomes while the latter are located on sex chromosomes and are called sex linked traits (sex linked inheritance). The genes that are carried by sex chromosomes through generations are called sex linked genes and the inheritance determined by the genes is termed sex linkage or sex linked inheritance. The phenomenon of sex-linkage is exemplified by the following.

A. X-linked genes:

Most of the sex linked genes are located on the X-Sex chromosomes. e.g *Drosophila*, man, poultry and other similar unisexual organisms. In the above given examples in the male individuals, the pair of sex chromosomes is heteromorphic i.e; one X Chromosomes and one Y chromosomes(XY), while it is homomorphic (XX) in female. However, this situation is reserved in birds where the female heteromorphic pair of sex chromosomes (i.e., ZW) and males have homomorphic pair (ZZ).

In males the single X- linked dominant or recessive gene expresses the trait phenotypically, while in female two X- linked genes are necessary for the determination or expression of a single phenotypic trait or character related to sex. The recessive X linked genes have characteristics crisscross inheritance (i.e., when male, for example, transmits his X –linked recessive genes to his grandson in F_2 generation through his daughter of F_1 generation).

1. Sex- Linkage in *Drosophila* :

In *Drosophila melanogaster* over 100 sex linked character have been identified. These affect every part of the body i.e.; eye color, body color with size, wing venation, eye shape and distribution of abdominal marking, etc. They are exactly like autosomal characteristics, usually they occur in both sexes an exhibit in offspring and depend solely upon the fact the factors are borne in the sex chromosomes.

The inheritance of X-linked genes in *Drosophila* (Eye color) takes place following methods.

I. White eye female X Red eye male: T.H. Morgan (1910) found the first X linked gene in *Drosophila.* This was the recessive white eye mutant gene. When a white eyed female (ww; recessive) crossed to a red eyed male (W, dominant), all the females are red eyed and all the males are white eyed in the F_1 generation. When these females and male of F_1 generation are

inter crossed, the female population will consist of 50% red eyed and 50% white eyed in the F_2 generation. Similarly the males will be 50% red eyed and 50 % white eyed in this generation.

II. **Red eye female X white eyed male:** Likewise in other experiment, when a red eyed female (WW) is crossed with a white eyed male (w), the males and the females would be red eyed in the F_1 generation. When males and females of F_1 generation are inter crossed, the female would be exclusively red eyed, however, in the male 50% would be red eyed and 50 % white eyed in the F_2 generation.

Ultimately, Morgan and others interpreted this to mean that the white-eye locus was on the X chromosome. Thus we see in the above reciprocal experiments that the character (located the X-chromosomes) is always transferred from the mother to the son and never from father to the son.

2. Sex -linkage in man:

In human about 56 sex linked genes have been identified. The most common X-linked genes of man are such as color or red green blindness, night blindness myopia (short sightedness), hemophilia, juvenile, glaucoma (hardening of eyeball) hidrotic ectodermal dysplasia, HED (non-functional sweat glands) and white forelock, etc. These diseases are dependent upon the X-linked recessive genes. So, sex-linked character in man will also follow the same pattern as in *Drosophila*.

The transmission of X-linked traits is in a zigzag manner. Females transmit X chromosomes to both sons and daughters. Males transmit the X chromosomes only to daughters and Y chromosomes to sons. The X-linked traits, which are recessive, are preferentially seen in males, who are always hemizygous for the X chromosomes. Females are heterozygous and form the "carriers" of that trait. Most X-linked traits are recessive.

I. **Color Blindness in man:** Some men are not able to distinguish red and green colors in the spectrum. They are said to be red and green color blind. The color blindness gene is located on the X-chromosomes. This gene plays an important role in the synthesis of so many substances in the color sensitive cell of retina. The genes for colorblindness are recessive to its normal allele and denoted by 'c'. The inheritance of color blindness can be studied by following two types of marriages.

(i) Color Blind female X normal vision male: When the color blind female (X^cX^c) marries a normal male (X^+Y) males would be color blind and females will be normal vision in the generation. It is because males receive one X linked recessive gene for color blindness from color blind mother. However, the male does not carry the gene for color blindness, denotes ad '+', the normal allele of 'C' in his chromosomes 'X'. The 'Y' chromosomes are inert i.e., having no allele for this gene. So his genotype is X^+Y or X^cY. The females, therefore, receive a X^+- linked dominant gene for normal vision from father and one X^c linked recessive gene for color blindness from the mother. Such females are referred to as carriers for colorblindness.

If the carrier females marry with normal men, they produce a color blind homozygous female, a normal visioned heterozygous female, a normal vision hemizygous male and a hemizygous color blind male in the F_2 generation.

(ii) Normal vision female X color Blind male: Likewise, in another experiment, when a normal vision female (X^+X^+) marries with a color blind male (X^cY), all the males would be normal with X^+Y and all the females would be carriers with genotype X^+X^c , in the F_1 generation . Thus, in the F_2 generation, two normal vision females, one normal vision male and one color blind male would be borne.

Thus the gene for color blindness from a male passes on to his grandsons (males) through his daughters (females). Thus inheritance of human color blindness is of crisscross type.

II. Hemophilia in man: It is also a sex linked recessive trait. In this disease, the blood coagulation inhibited, therefore in the event of an injury, the patient bleeds continuously the last drop of the blood is drained out. John Cotto (1803) reported this hereditary disease first of all in man. This disease was most common in royal families of Russia and Queen Victoria of England. Queen Victoria was a carrier for this haemophilic trait and among her four sons Leopold was hemophilic.

III. Night Blindness in man: Some people suffer from night blindness disease. They experience difficulty in viewing in dim light. This trait is also a sex linked recessive one.

B. Inheritance of Y-linked or holandric genes:

Men are homozygous for Y-linked genes present on the non-homologous parts. All these genes will be expressed in all conditions. These genes are always transmitted from father to sons and never to daughters. There are no essential genes in the Y chromosome except the locus for the maleness and fertility. In man, Y-linked gene (*Ichthyosis hystrix* Gravior, hypertrichosis i.e., excessive development of hairs on pinna or ear, etc.) is inherited directly from father to son.

C. Inheritance of XY-linked Genes:

There are some homologous regions in the X chromosomes and Y chromosomes. These homologous parts pair during meiosis and may undergo crossing over. Therefore, genes in these homologous regions show inheritance similar to autosomal genes and are called pseudoautosomal inheritance. The gene located in homologous section of 'X' and 'Y' chromosomes termed as XY-linked genes. Such genes or characters are very rare. e.g; *Drosophila melanogaster*, an XY-linked gene i.e., bobbed gene for bobbed bristles located in the X –Chromosomes or its allele may be located in the Y-Chromosomes (recessive). This bobbed allele (bb) is cytologically positioned in the neighbourhood of the nucleolus organiser NO.

In human, several diseases like total color blindness, skin pigmentation a cancerous disease (Xeroderma pigmentosum), Epidermolysis bullosa , Retina pigmentosa **(**degeneration of retina due to deposition of pigment in the eye **),** Nephritis **(**a kidney disease**)** are XY-linked.

2. Sex-linkage in plants:

Some plants like corn and garden vegetables have both sexes occurring in same individual. There is no sex difference between individuals and no sex chromosomes exist. So no sex linkage is

possible in such plants. However in some bisexual plants like hemp, date palms and willows, sex chromosomes have been identified and hence, sex-linkage in known. *Silene latifolia* (Lychnis) also exhibit sex-linkage. A mutant type having narrow rosette leaves of Lychnis had been discovered. When this mutant was crossed with the normal broad leaved type, following result were obtained.

P_1 Broad leaf X Narrow leaf
F_1 Broad + Broad
F_2 Broad : 1 Broad : 1Narrow
Hence, narrow is a typical sex-liked recessive trait in *S. latifolia* (Lychnis)

9.6 SUMMARY

Sex determination explained that sex chromosome play a great role in heredity. Each individual has two sex chromosomes; females have two X chromosomes (XX), while males have one X chromosome and one Y chromosome (XY). Sex-linked traits are located on genes on the sex chromosomes. Experiment by Morgan and Bridges have proved that sex chromosomes have special genes which are not found in autosomes (somatic chromosomes). By observations of Wilson it has been clear that both the heterogamic sex chromosomes differ in structure and types of genes. Various genes which are present in sex chromosomes are known as sex linked genes. The characters governed by the sex linked genes are called sex linked characters. Transmission of such sex linked characters from one generation to another is known as sex linked inheritance.

Sex determinations have lot of importance in horticulture, crop culture and poultry. Several fruits and crops are monosexual and also bisexual. It is important to identify the female and male sexes. Similarly in poultry it is also essential to identify male and female at the time of birth.

9.7 GLOSSARY

Alleles: Alternative forms of a gene or DNA sequence occurring at the same locus on homologous chromosomes.
Allosome: Sex chromosomes can also be called as allosome.
Autosome: Any chromosome other than the sex chromosomes.
Carrier: A healthy person possessing a mutant gene in heterozygous form: also refers to a person with a balanced chromosomal translocation.
Chromosome: A structure within the nucleus composed of double stranded DNA bearing a linear array of genes that condenses and becomes visible at cell division.
Complete linkage: Inheritance patterns for two genes on the same chromosome when the observed frequency for crossover between the loci is zero.
Diocious: If male and female flowers are present on different plants then it is dioecious.
Diploid: It is that organism which has two sets of chromosomes. Zygote is a single celled diploid.
Dominant Trait: expressed in people who are heterozygous for a particular gene.

***Drosophila melanogaster*:** The fruit fly, a favourite organism for genetic analysis.

F_1: Its full name is first filial generation. It is the first generation obtained by hybridization.

F_2: It is the second generation obtained on self fertilization of F_1.

F_3: It is the third generation obtained on selfing of F_2 hybrids.

Gamete: Egg or sperm. Cells formed after meiosis are called gametes. A Gamete is a single celled haploid.

Gene: A gene is a basic and functional unit of heredity. It is composed of DNA and act as instructions to make a protein molecule. Genes are located on chromosomes.

Genetics: The field of biology that studies genes, genetic variation and heredity in living organisms.

Genome: Total DNA compliment of a gamete or a haploid cell.

Genotype: Genetic constitution of an individual organism.

Hemizygote: Organisms having only one copy of a gene in diploid cells (in human males are hemizygous for most X linked genes).

Heredity: The passing on of phenotypic traits from parents to their offspring, either through sexual or asexual reproduction. Offspring cells or organisms are said to inherit the genetic information of their parents. It is also called inheritance.

Heterozygote: An organism possessing different alleles at a particular locus on homologous chromosomes.

Holandric: Pattern of inheritance of genes on the Y chromosome.

Homologous: Chromosomes that pair at meiosis and contain the same set of gene loci. Organisms have two identical alleles for a given gene at a particular locus on homologous chromosomes; both alleles at corresponding loci are identical.

Hybrid: Hybrid is the offspring of parents of different genotype. It results from a cross between two races, breeds, strains or varieties of same species.

Karyotype: An array of all the chromosomes found in a cell of an individual. Typically the chromosomes are stained to reveal size, banding pattern, or other distinguishing feature to enable the identification of any abnormalities.

Linkage: Genes that are inherited together on the same chromosome.

Locus: Site of a specific gene or DNA sequence on a chromosome.

Meiosis: Cell division during gametogenesis resulting in haploid gametes. Here the number of chromosomes is reduced to half producing gametes.

Non-linkage: Non-linkage describes the inheritance patterns for two genes on the same chromosome, when the expected frequency for crossover between the loci is at least one. The observed inheritance pattern for non-linked genes on the same chromosome is the same as for two genes on different chromosomes.

Partial linkage: Partial linkage describes one of the inheritance pattern for two genes on the same chromosome, when the expected frequency for crossover between the loci is greater than zero but less than one. From partial linkage analysis we can learn about the order and spacing of genes on the same chromosome.

Phenotypic/phenotype: The observable or physical characteristics of an individual, as a result of genetic expression and environment.

Recessive: An allele that produces its characteristic phenotype only when the paired allele is the same; will be masked if a dominant allele is present. Trait expressed in people who are homozygous or hemizygous for a particular gene, but not in those who are heterozygous for the gene.

Recessiveness: A relationship between the alleles of a gene in which one allele produces an effect on phenotype that is overpowered or "masked" by the contribution of another allele at the same locus; the first allele and its associated phenotypic trait are said to be recessive, and the second allele and its associated trait are said to be dominant. In genetics shorthand, recessive alleles are often represented by a lowercase letter (e.g. "a", in contrast to the dominant "A").

Sex chromosomes: Sex determination is based on sex chromosomes

Sex-linked inheritance: Inheritance of traits that are encoded for in genes on the sex chromosomes.

Sex-linked trait: Trait linked to genes located on the sex chromosomes.

Sex linked: A gene coded on a sex chromosome, such as the X-chromosome linked genes of flies and man.

Single-Nucleotide Polymorphism (SNP): Genetic variation in a DNA sequence that occurs when a single nucleotide in a genome is altered; SNPs are usually considered to be point mutations that have been evolutionarily successful enough to recur in a significant proportion of the population of a species.

X chromosome : A gene or DNA segment located on the X chromosome.

Y chromosome: One of two sex chromosomes present in organisms which use the XY sex-determination system. The Y chromosome is found only in males and is typically much smaller than its counterpart, the X chromosome.

Zygote: It is a cell formed by fertilization of female gametes by a male gamete. The cell formed when two gametes (sperm and egg) are fused via sexual reproduction; earliest stage in embryonic development.

9.8 SELF- ASSESSMENT QUESTIONS

9.8.1 Fill in the blanks:

a) Alternative forms of genes are called _____.
b) Humans have _____ pairs of chromosomes.
c) What type of allele will be expressed if both dominant and recessive alleles are present for a given trait?
d) Males tend to inherit more sex-linked conditions because _____
e) The expression of genes is called the _____.
f) What is the probability of having a child with a recessive trait if both parents are heterozygous for the trait?
g) Genes that are located on the same chromosome are said to be _____

h) If a male inherits a sex-linked gene for color blindness _____.
i) A person without a Y chromosome will _____.
j) Heterozygous parents who have had one child with a recessive disease will still have a _____ chance of their second child being born with the same recessive disease.

9.8.2 Multiple choice questions:

1. If a gene is found on X chromosome only than it is said to be:
(a) X-linked (b) Y-linked
(c) Sex-linked trait (d) XY linked

2. Sex-linkage is linked to the:
(a) Gender of organism (b) Skin color of organism
(c) Body shape of organism (d) limb structure of organism

3. Sex-linked traits include:
(a) Color blindness (b) Male pattern baldness
(c) Haemophilia (d) all of above

4. Hemophilia is a sex-linked recessive trait in humans. If a father and a son are both hemophiliacs, but the mother is normal, her genotype must be:
(a) X^hX^h (b) X^HX^h
(c) X^HX^H (d) X^hY

5. Which of the following gives information about the phenotype but not the genotype?
(a) X HY (b) Hemophiliac man
(c) Tall pea plant. (d) Female carrier for color-blindness

6. If two white sheep produce a black offspring, the parent's genotypes for color must be:
(a) Heterozygous (b) Homozygous white
(c) Homozygous black (d) Not enough information was given

7. In *Drosophila* (fruit flies), eye color is sex-linked and red eye color is dominant to white eye color. Which of the following are not possible in a cross between a red-eyed male and a heterozygous female?
(a) Red-eyed male (b) White-eyed male
(c) Carrier female. (d) Homozygous white-eyed female

8. Carriers of the color-blindness trait include:
(a) Men who are heterozygous for the trait.
(b) Men who are homozygous for the trait.
(c) Women who are heterozygous for the trait.
(d) Women who are homozygous for the trait.

9. Normal human eggs have:

(a) 22 autosomes and an X chromosome (b) 22 autosomes and a Y chromosome.
(c) 23 autosomes (d) 46 chromosome

9.8.1. Answers Key: (a) alleles, (b) 23 pairs, (c) dominant, (d) there is no corresponding allele on their Y chromosomes, (e) genotypes, (f) 25 %, (g) Linked, (h) will always be expressed, (i) always show female characteristics, (j) 25 %

9.8.2 Answers Key: 1-(c), 2-(a), 3-(d), 4-(b), 5-(c), 6-(a), 7-(d), 8-(c), 9-(a)

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9.10 SUGGESTED READINGS

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9.11 TERMINAL QUESTIONS

9.11.1 Very Short Answer Type Questions:

a) Define incomplete linkage.
b) What is recombination?
c) What is X-linked Inheritance?
d) Write about Sex influenced characters.
e) Explain about Color blindness in human
f) Write about Hemophilia
g) Define Gynandromorphs.
h) Write about Heterogametic sex
i) Differentiate between Sex chromosomes and autosomes
j) How do autosomal traits differ from sex linked traits?
k) What are the sex chromosomes of a male and a female?
l) Explain why the father determines the sex of the child.
m) What is sex-linked inheritance?

n) Define sex-linked trait.
o) A son cannot inherit color-blindness from his father. Why not?

9.11.2 Short Answer Type Questions:

a) Explain the mechanism of sex determinations.
b) What is sex linked gene? Give examples.
c) Illustrate sex linked inheritance with a suitable example.
d) Elaborate Linkage and Crossing Over.
e) What is Gene-linkage? How is it different from the Sex-Linkage?
f) Explain the genetics of sex determination in humans.
g) Write a notes on :
   XX-XY type of sex determinations,
   XX-XO type of sex determinations,
   ZW-ZZ type of sex determinations
   ZO-ZZ type of sex determinations
h) Hemophilia in man is due to an x chromosomes mutation. What will be the results of mating between a normal (non-carrier) female and hemophilic male?
i) A human female "carrier" who is heterozygous for the recessive sex linked trait causing red green color blindness (or alternatively hemophilia) marries a normal male. What proportion of their male progeny will have red green color blindness (or alternatively, will be hemophiliac).

9.11.3 Long Answer Type Questions:

1. Describe type of linkage with examples.
2. What is Sex Linked Inheritance? A hemophiliac woman has a mother who is phenotypically normal. What are the genotypes of her parents?
3. Illustrate Multiple Alleles with ABO blood group as an example. A woman homozygous for blood type B marries a man who is heterozygous for blood type A. State the possible phenotypic ratio of the offspring.
4. Red green color blindness in human is recessive and sex linked. If a woman heterozygous for color blindness marries a color blind man, what is the probability that their first child will be a color blind daughter?
5. The gene for yellow body color **y** in *Drosophila* is recessive and sex linked. Its dominant allele y^+ produces wild type body color. What phenotypic ratio are expected from the crosses given below?
   (a) yellow male X yellow female
   (b) yellow female X wild type male
   (c) wild female (homozygous) X yellow male
   (d) wild type (carrier) female X wild type male
   (e) Wild type (carrier) female X yellow male.
6. Differentiate between the following, giving location of genes, mode of transmission and relationship of sex:

(a) Sex influenced and holandric character,
(b) Sex linked and sex influenced characters.

7. In a cross between white eye female fruit fly and red eye male, what percent of the female offspring will have white eyes? (White eyes are X-linked recessive).
8. In a cross between a pure breed, red eye female fruit fly and white eye male, what percent of the male offspring will have whit eyes (white eyes are X linked recessive).
9. A human female "carrier'' who is heterozygous for the recessive sex limited trait red colour blindness, marries a normal male. What proportion of their female progeny will have red green color blindness or alternatively will be hemophilic?

UNIT-10 LINKAGE AND CROSSING OVER, MUTATION

10.1 OBJECTIVES

After reading this unit students will be able-

1. To understand the basic process of genetic inheritance.
2. To analyze the actual facts and mechanism working behind inheritance of characters between progenies.

10.2 INTRODUCTION

The genes are the hereditary units. The genes are responsible to determine the characters of an individual and are located on the chromosomes. An individual usually have many genes for the determination of different characters. Naturally there are more genes than the number of chromosomes. Thus it can be expected that each chromosome contains more than one gene. Sutton (1903) suggested the Chromosomal Theory of Inheritance. The genes are responsible for the expression of characters. The genes for different characters may be either situated either on the same chromosome or in different chromosomes. If the genes are situated on different chromosomes, the character they control may appear in next generation either similarly or differently depending upon the chances of gametogenesis. Because as any organism has many more genes then number of chromosomes, in each chromosome there are many loci and the genes are arranged in loci in a linear fashion on chromosome like beads on a string/ thread. This concept was demonstrated for the first time by Sturtevant (1913).

These genes are assorted independently according to Mendel's Law of Independent Assortment. When genes for different characters are located on the same chromosome, they are tied to one another and are said to be linked. After the discovery of Mendel's unique work, a number of biologists were involved to conduct experiments of Mendel's conclusion on different groups of plants and animals. During this period although most of the conclusions were same yet after some time a large number of abnormalities were studied in the behavior of characters which were not covered by Mendelian laws, in some cases investigated abnormalities were studied in the behavior of characters which were not governed by Mendelian laws. In some cases investigation did not confirm to the law of dominance and law of independent assortment so some modifications of them became necessary.

10.3 LINKAGE

It is evident from studies that gene is the hereditary unit and determine the characters of an individual. The genes are carried by the chromosome. To determine various characters one individual has many genes. It means the number of genes is more than the number of chromosomes, hence it can be expected that each chromosome bears more than one gene. These genes are responsible for the expression of characters, for this there may be one gene or more than one gene to express a single character in the individual. The genes for different characters may be either situated in the same chromosome or on different chromosomes. Thus the character

they bear or control, appear in next generation either together or apart depending upon the situation. When the genes are situated on different genes and control a single character these genes assort independently according to Mendel's law of Independent Assortment. But contrary to this if the genes are situated on same chromosome and are fairly close to each other they have a tendency to inherit together in the next generation. This special type of coexistence of two or more genes in the same chromosome is known as "Linkage". It is understood that linkage is a modification of Mendel's law of independent assortment in which the non- allelic parental combinations may not necessarily continue as such (as in parents) in future generations or in offspring. However, there is great difference in Independent assortment and Linkage. The difference between these two can be understood by the following example:

Example 1- When genes situated on different chromosomes they assort independently and gives 1:1:1:1 test cross ratio with four different characters. This shows independent assortment.
In a dihybrid cross there are parents AABB (dominant) and aabb (recessive). After crossing they produced AaBb (dominant) generation in F_1 generation. This F_1 generation when test crossed it gives 1:1:1:1 test cross ratio, shows independent assortment.

P_1 AABB (dominant) (male) X aabb (recessive) (female)

P_1 gametes- (AB) (ab)

F_1 generation- (AaBb) (dominant)

Test cross- AaBb (male) X aabb (female)

Gametes = AaBb= AB, Ab, aB, ab X ab

F_2 generation- ¼ AaBb: ¼ Aa bb: ¼ aaBb: ¼ aabb or 1:1:1:1 Test cross ratio.

Example 2- The linked genes associate together so they do not assort independently. They stay together in the same combination in next generation as in their parental one. To show linkage genes on one chromosome shows to the left of the slash (/) and on right are on homologous chromosome. They show as AA/BB (dominant) and aa/bb (recessive)

P_1- AA/bb (male) X aa/bb (female)

P_1 gametes- AB ab

F_1 generation- AB/ab (dominant)

Test cross= AB/ab (male) X ab/ab (female)

Test cross gametes- (AB) (ab) (ab)

F_2 generation- ½ AB/ab : ½ ab/ab or 1:1 ratio.

Historical Aspect of Linkage- T. H. Morgan in 1911 proposed a hypothesis for this phenomenon that those genes linked together tend to remain in their original combinations because of their location on the same chromosome. He was working on famous fruit fly

(*Drosophila melanogaster*). His material was easy to culture in laboratory, easy to handle, having short life span and very suitable material for experimental point of view. But prior to Morgan, Sutton (1903) and Bateson and Punnett (1906) had already given some idea of the phenomenon of linkage. However, Mendel could not notice the phenomenon of linkage because the seven pairs of alleles or characters studied by him in pea plant were located in seven different pairs of chromosomes. Various geneticists expressed their view about this phenomenon from time to time as follows:

1. Sutton' View on Linkage- i) The chromosomal theory of inheritance was proposed by Sutton and Boveri states that chromosomes are vehicles of genetic heredity whereas linkage causes alleles on the same chromosome to be inherited together. Sutton stated that genes are arranged on chromosomes in linear order. During meiosis chromosomes moves as units so all the genes of a chromosome are linked. According to him, genes are the units of heredity and situated on chromosomes. As the numbers of characters are more than the number of chromosomes thus each chromosome may contain a number of genes. During meiosis the chromosomes moves as units therefore, all the genes which are situated in same chromosome or on one chromosome will be linked together. Since as a result of those particular linked genes each species of animals or plants would have a specific number of group of linked genes which would correspond with the number of chromosomes found in that particular species. Sutton could not succeed to prove his hypothesis experimentally.

2. Bateson and Punnett's Hypothesis- Bateson and Punnett (1906) when experimenting Mendel's law of Independent Assortment in a sweet pea plant observed the unexpected F_2 results of a dihybrid cross between a homozygous sweet pea (*Lathyrus odoratus*) plant having dominant alleles for blue color flowers (BB) and long pollen grains (LL) with another homozygous double recessive plant with red flower color (bb) and round pollen grains (ll). The F_1 individual (BbLl) with blue flower and long pollen grains were obtained. When this F1 was test crossed i.e., blue long (BbLl) with double recessive plants (bbll) having red flower and round pollen grains, they observed unexpected phenotypic ratio of 7:1:1:7 instead of the expected ones 1:1:1:1. The details are illustrated as follows-

P_1 generation- Blue Long X Red Round (Phenotype)

(BBLL) (female) (bbll) (male) (Genotype)

P_1 Gametes (BL) (bl)

F_1 Generation (BbLl) (Blue Long)

Test Cross F_1 Blue Long X Red Round

(BbLl) (female) (bbll) (male)

Test Cross Progeny- 7/16 blue Long (BbLl): 1/16 Blue Round (Bbll:1/16 Red Long (bbLl):

7/16 Red Round (bbll). (7:1:1:7).

The 7:1:1:7 test cross ratio instead of 1:1:1:1 shows that there was a tendency in the dominant alleles to remain together. This deviation was explained as "Gametic Coupling" by Bateson and Punnett. It is observed that when two such alleles either dominant or recessive come from different parents, they tend to remain separate. They termed it as repulsion. To prove this, Bateson and Punnett considered another test cross of sweet pea plant having blue flower and round pollen (BBll) and another plant with red flower and long pollen (bbLL) or heterozygous parents and obtained the ratio of 1:7:7:1 in F_2 generation illustrated in following figure-

P_1 Generation- Blue Round X Red Long (Phenotype)

(BBll) (male) (bbLL) (female) (Genotype)

P_1 Gametes- (Bl) (bL)

F_1 Generation- Blue Long (BbLl)

Test Cross- F_1 Blue Long X P_1 Red Round

BbLl (male) bbll (female)

Test cross Progeny- 1/16 Blue Long (BbLl):7/16 Blue Round (Bbll):

7/16 Red Long (bbLl): 1/16 Red Round (bbll). Or 1:7:7:1.

Explanation: i) The 7:1:1:7 test cross ratio clearly indicated that there was a tendency in the dominant alleles (BBLL) to pass together/inherit together to the same gamete. Similarly in the case of recessive alleles (bbll) this tendency of dominant and recessive alleles to transfer together was termed as "gametic coupling" by Bateson and Punnett.

ii) The 1:7:7:1 in this test cross hence the two dominant alleles repelled each other, the tendency of both dominant and recessive alleles to repel each other, so that the gametes of (BBll) or (bbLL), (BBll) are formed more frequently and they termed it as gametic repulsion.

Bateson and Punnett were unable to explain this phenomenon of coupling and repulsion theory.

T. H. Morgan in 1910 explained this phenomenon when working with *Drosophila.* Morgan noticed that coupling or repulsion was not complete. According to Morgan the two genes of a parent are either in coupling phase or in repulsion phase. When they are present on the same chromosome then it shows coupling and when present on two different homologous chromosomes then it shows repulsion. Such genes are called linked genes and this phenomenon of inheritance of linked genes is called linkage by Morgan.

Morgan explained coupling and repulsion on the basis of pairs of gametes present in homozygous or heterozygous parents. The probabilities are that the pairs of genes of homozygous parent end to enter in same gamete and remains together or, same genes from heterozygous parents tend to enter in different gametes and remain apart from each other. According to him the linked genes shows a tendency of remaining together in original combination due to their close location in the same chromosome. However, the degree or

strength of linkage depends upon the distance between the linked genes in the same chromosome. This concept of linkage genes given by Morgan latter developed into the famous theory of linear arrangement of genes in the chromosome which helps in the construction of genetic or linkage maps of chromosome.

Later this term was changed as "Cis" and "Trans" by Haldane (1942) in which the former replacing coupling and the later, repulsion (Fig.10.1).

Cis and Trans Arrangement of genes: The linked genes show two types of arrangement on the chromosomes. If the dominant alleles of two pairs of linked genes are present on one chromosome and their recessive alleles of all of them on the other homologue (AB/ab), this arrangement is known as Cis arrangement. However, if the dominant allele of one pair and recessive pair of second pair are present on one chromosome and recessive and dominant alleles on the other chromosome of a homologous pair (Ab/ab), this arrangement is called Trans arrangement.

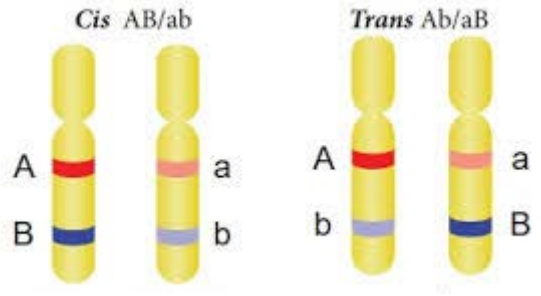

Fig. 10.1 Cis and Trans arrangement of Genes

10.3.1 Types of Linkage

On the basis of Morgan and his co-workers findings on *Drosophila* and other organisms, there may be two types of linkage:

1. Complete Linkage or Perfect Linkage and
2. Incomplete Linkage or Partial Linkage.

1. Complete Linkage- When parental combinations of characters appear together for two or more generations in a regular and continuous fashion. The phenomenon is known as complete linkage. In this case the genes are associated closely and hence transmit together. It is rare.
Example- In case of *Drosophila melanogaster* its fourth chromosome mutant having genes for bent wings (bt) and shaven bristle (sv) is the example of complete linkage.

2. Incomplete Linkage- When the genes on the same chromosome do not remain linked or together because during meiotic prophase the homologous non- sister chromatids may exchange segments of different lengths with one another. This phenomenon is known as crossing over in which the linked genes widely located in chromosomes and have chances of separation by crossing over is also called incomplete linked genes and the phenomenon of their inheritance of genes is called incomplete linkage. Incomplete linkage is frequent in nature and has been

reported in various organisms such as poultry, mice, man, pea, tomato, maize and even in *Drosophila.*

Example- The best example of this type linkage was put forward by Morgan in case of wild female *Drosophila.* Besides this in many other organism viz., peas, maize, tomato among plants and, mice, poultry and man among animal kingdom are such examples. A few of them are reported here for instance; in maize both the sexes have shown recombination between linked genes i.e., a cross between two varieties of maize, having colored and full seeds (CS/CS) and another with colorless and shrunken seeds (cs/cs). In this case, Hutchinson observed incomplete linkage between the alleles for color and shape of the seed. When a maize plant with colored seeds and full endosperm (CS/Cs) crossed with another plant having colorless seeds and shrunken endosperm, the previous one acts as dominant while the later one displays recessive alleles. In F_1 offspring with colored full (phenotype) and CS/CS genotype was obtained. When this F_1 hybrid is test crossed with double recessive parents (cs/cs) instead of two, four types of offspring were obtained as shown in following figures:

Parents P_1- Colored full X Colorless shrunken (Phenotype)

CS/CS (male) ↓ cs/cs (female) (Genotype)

F_1 generation Colored full

(CS/cs)

Test cross- F_1 colored full X Colorless shrunken (double recessive)

CS/cs (male) ↓ cs/cs (female)

Test cross results- Colored full: Colored shrunken: Colorless full: Colorless shrunken

CS/Cs	Cs/cs	cS/cs	cs/cs
40%	2%	2%	40%

Explanation- Thus the above results, clearly showing 96% of alleles having parental combinations are expected from complete linkage, while the other two the new combination Cs/cs and cS/cs in 4% cases. It is obvious that in 4% cases only crossing over have occurred between linked genes.

Another example of incomplete linkage was reported in wild type *Drosophila* having different body color and wing shapes. If a grey body and long winged *Drosophila* with genotype ($b^+v^{+/}b^+v^+$) , where alleles for grey color b+ and long wing v+ are dominant over the mutant alleles for black color b and vestigial wing v. A dominant fly having grey color and long wing (b^+v^+/b^+v^+) is crossed with a recessive one with black body and vestigial wing (bv/bv). In F_1 generation heterozygote (b^+v^+/bv) with grey body and long wing genotype is found. When F_1 is

test crossed with double recessive parents (bv/bv) interesting results are obtained. Beside the parental combination (83%) 41.5% each, two new combinations (17%) 8.5% each were obtained. Thus in 17% crossing over has occurred while 83% have shown linkage. So it does not display 1:1 test cross ratio as shown in following figure:

Parents P_1 Grey Long X Black Vestigial (Phenotype)

$b^+v^{+/}b^+v^+$(male) ↓ bv/bv (female) (Genotype)

F_1 generation- Grey Long

$b^+v^{+/}bv$

Test cross- F_1 Grey Long (male) X Black vestigial (female) (Double recessive)

↓

Gray Long: Grey Vestigial: Black Long: Black Vestigial

b^+v^+/b^+v^+: b^+v/bv: $bv^{+/}bv$: bv/bv

41.5% 8.5% 8.5% 41.5%

In this case 83% alleles exhibited complete linkage and showing parental combination with 41.5% each while 17% alleles revealed crossing over and expressed new combinations- Grey vestigial (b^+v/bv) 8.5% and Black Long ($bv^{+/}bv$) 8.5%, thus showing crossing over in 17% alleles.

Linkage Group: All the linked genes of a chromosome form a linkage group because all the genes of a chromosome have their identical genes or allomorphs on the homologous chromosome. Therefore, linkage group of a homologous pair of chromosome is considered as one. Thus the number of linkage groups of a species corresponds with haploid chromosome number of that species.

In genetics, all the genes on a single chromosome are known as linkage group. They act as one and move as a unit rather than independently. The total number of linkage group in an organism corresponds to the number of chromosome pairs. For example, there are 23 linkage groups in man, 7 in sweet pea, 4 in *Drosophila malenogaster* and 10 in *Zea mays*, etc**.**

Significance of Linkage: Linkage is very significant as it plays an important role in determining the nature of scope of hybridization and selection program. Linkage reduces the chance of recombination of genes and thus helps to hold parental characteristics together or, it helps to maintain its parental, racial and other characters. Linkage limits the variability among individuals.

10.4 CROSSING OVER

In the previous chapter, we have studied that the genes located in the same chromosome and situated quite close to each other show complete linkage and they inherited together in the same manner in the next generation or they may be segregated or separated during gametogenesis when studied widely in a chromosome. These genes show incomplete linkage. The incomplete linkage takes place due to the occurrence of new combinations or recombinations of linked genes. The recombination is accomplished through a process known as "Crossing Over", in which the non sister chromatids or segments of chromosomes of homologous chromosomes exchange the chromosomal parts or segments. Crossing over is the exchange of genes between two chromosomes, resulting in non- identical chromatids that comprise the genetic material of gametes. This process occurs during Prophase I of Meiosis, just prior to chromosome alignment and splitting of the cell.

Crossing over is the exchange of genetic material between non- sister chromatids of homologous chromosomes during meiosis, which results in new allelic combinations in the daughter cells.

The crossing over is the process that produces new combination or recombination of genes by interchanging of corresponding segments between non-sister chromatids of homologous chromosomes. The term crossing over was first used by Morgan and Cattell (1812). The exchange of precisely homologous segments between non-sister chromatids of homologous chromosomes was performed. After exchange of chromosomal parts resulted chromatids are known as "Cross Overs". On the basis of its occurrence in somatic or germ cells there are two types of crossing over as following:

1) Somatic or mitoic crossing over- The crossing over occurs in somatic or body chromosomes during mitotic division then it is known as somatic or mitotic crossing over. The somatic crossing over is rare and has no genetic significance. Curt Stern in *Drosophila* and G. Potnecarvo in fungus *Aspergillus nidulans* also reported somatic crossing over in somatic or body cells.

2) Germinal or meiotic crossing over- Crossing over is generally common in germinal cells at the time of gametogenesis (during meiosis) and so is called as meiotic or germinal crossing over. The meiotic crossing over is universal in its occurrence and is of great genetic significance. The genetic or linkage maps are constructed on the basis of crossing over.

Mechanism of Crossing over- It occurs due to the interchange of sections of homologous chromosomes. The chromosomes usually undergo breakage during gametogenesis. Thus a mechanism does exist by chromosome by which group of genes on the same chromosome changes with the similar group of genes on the homologous chromosome.

The process of crossing over includes the following stages-

1) Synapsis. 2) Duplication of chromosomes. 3) Crossing over and 4) Terminalization.

1) Synapsis- In nature sex cells, the homologous chromosome come close to each other and pairing starts during zygotene stage of Prophase I of meiosis. It is called synapsis. Synapsis is an event of prime importance in meiosis which provides the mechanical basis of heredity and variation. It starts during zygotene when chromosomes are held to make contact with each other at one or more points from which synapsis extends into adjacent regions and it ends or reaches its maximum in pachytene after which the homologous chromosomes get separated from each other except the regions of chaismata. Thus synapsis is a prolonged phase in which the two homologous chromosomes (due to attraction between these chromosomes) come close to each other at two exactly identical or homologous regions. These paired homologous chromosomes are now known as "bivalents".

Many geneticists proposed different theories about the process of synapsis. Darlington (1937) proposed precocity theory of meiosis. The theory was experimentally verified by Hotta and others in 1966. Cyrill Dean Darlington (1903-1981) was an English biologist and Eugenicist who discovered the mechanism of chromosomal crossover, its role in inheritance and therefore, its importance to evolution.

The theory of Darlington was experimentally verified only after 1955 when it was clearly proved that the main bulk of deoxyribonucleic acid or DNA of the total genome is already synthesized in the premeiotic interphase and is completed later by the leptoene period. Yasuo Hotta and others (1966) reported that the amount of DNA not synthesized in premeiotic mitosis in *Lilium* constitutes only 0.3% of total DNA, synthesized during zygotene or pachytene period by employing cytomaterial and autoradiographical investigations in meiosis.

Later in 1968 Yuri Bogdanov and his coworkers experimenting in *Gryllus domesticus* (house cricket) during spermatogenesis while studying synthesis of DNA and histone protein by applying quantitative cytophotometric techniques reported that DNA synthesis that convert the 2C into 4C amount occur prior to leptoene, while histone synthesis is delayed by 25% in zygotene and is completed only by pachytene. Thus chromosomes enter Prophase- I with almost complete synthesis of DNA but with incomplete synthesis of histone.

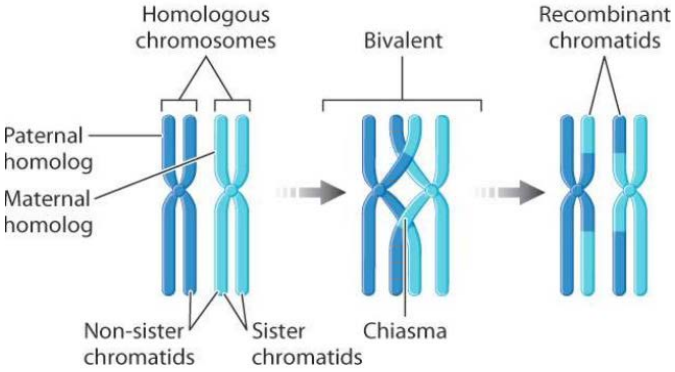

Fig. 10.2 Pairing of homologous chromosomes and crossing over

Thus based on these findings they proposed that the shortage of 0.3% DNA and 255 histone proteins in chromosomes during zygotene creates the condition for their homologous pairing. This hypothesis is a modernized version of Darlington precocity theory.

Now the question arises that why do homologous chromosomes approach each other during synapsis from a considerable distance and get closely associated. Darlington (1937) explained the cause of synapsis. According to him when chromosomes enter into meiotic prophase I, these consist of a single chromatid. Accordingly these non replicated chromatids are unbalanced or in an unsaturated electostatistical state. To become saturated or balanced the single stranded chromosomes must pair. Thus the sequence of meiotic pairing or synapsis is determined as per the precocity theory advocated by Darlington.

Synaptical complex- Synaptical complex (SC) is a protein structure that forms between homologous chromosomes during meiosis that forms two pairs of sister chromatids and is thought to mediate chromosomes pairing, synapsis and recombination. After the discovery of electron microscope, Montrose J. Moses in 1955 has revealed a highly organized structure of filaments in between the paired chromosomes of zygotene and pachytene stages in Crayfish and named it as Synaptical complex. However, after that the Synaptical complex has also been observed in a wide variety of species of plants and animals.

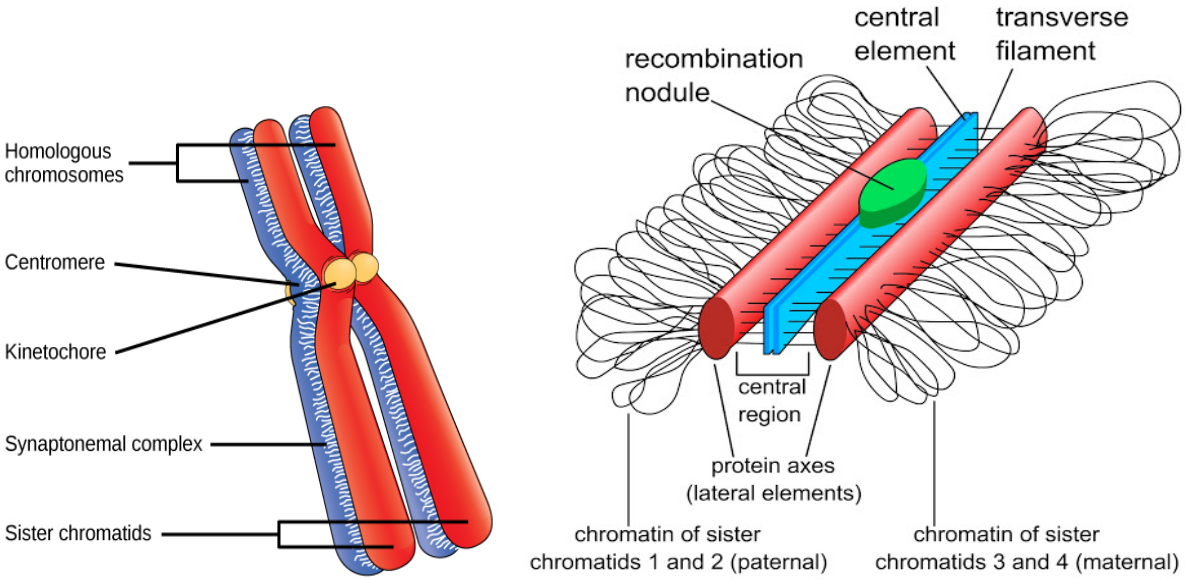

Fig. 10.3 Homologous chromosomes with synaptonemal complex and recombination nodule.

Structurally in electron micrographs the Synaptical complex appears as three parallel dense lines that lie equally spaced in a plane and are flanked by chromatin. Among these three parallel lines, the element of central line is of variable elements while the elements of two central lines usually appear dense. Some transverse strands also cross between lateral elements, connecting them with the central elements. Though the morphology of lateral and central elements may vary from species to species, but the basic structure and the spacing of the synaptonemal complex is constant within the species.

The synaptical complex contains DNA and a specific material, which is chemically proteinaceous in nature and known as synaptical complex material. Cytochemical studies of

synaptonemal complex have shown that there are differences in lateral and central elements as the lateral elements are rich in DNA, RNA and protein but the central one contains mainly RNA, protein and a little DNA.

Functions of Synaptical complex- Synaptical complex helps in chiasma formation and crossing over. The main functions of synaptical complex are following-

1) Maintenance of synapsis in fixed state for an extended period for crossing over to occur.
2) To provide a structural framework within which exchange of segments takes place.
3) To segregate recombination DNA from the rest of other chromosomal DNA.

Robert King (1970) studied the functions of synaptonemal complex and suggested that this may orient the non- sister chromatids of homologous chromosomes in a manner to facilitate exchange between their DNA. With the invention of electron microscopic studies, Comings and Okada (1971) reported that synapsis occurs at two levels; one at chromosomal level and other at molecular level. They revealed that the synatonemal complex pulls homologous chromosomes into approximate association with each other but plays no role in molecular pairing of DNA strand.

2) Duplication of chromosomes- In pachytene stage of meiosis the synapsis is followed by duplication of chromosomes. During this stage each homologous chromosome of bivalent splits longitudinally and forms two identical sister chromatids which remain joined together by an un splitted centromere. At this stage each bivalent contains four chromatids known as "tetravalent". The longitudinal splitting of chromosomes takes place by the separation of already duplicated DNA molecule along with certain chromosomal protein. Duplication is produced when extra copies of genes are generated on a chromosome.

3) Crossing over- It is a well known fact that crossing over occurs in the homologous non- sister chromosomes only during the four stranded or tetrad stage. The homologous chromosomes continuously remain in the synapsis stage during pachytene stage for a long period and then exchange of chromosomal material between non-sister chromatids of each tetravalent takes place through crossing over process. The recombination nodule becomes visible in pachytene stage between synapsed chromosomes.Stern and Hotta (1969) proposed a theory about the process of crossing over. According to them the two non- sister chromatids first break at the corresponding points due to the activity of a nuclear enzyme known as ***endonuclease.*** Thus a segment of one site of each tetravalent connects with a segment of the opposite site of the break.

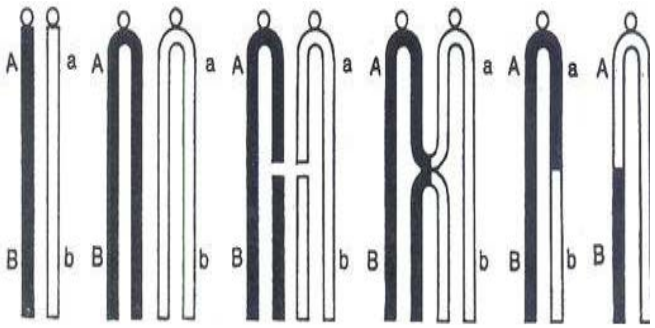

Fig. 10.4 Diagram showing mechanism of crossing over

The two non- sister chromatids cross each other. At this a little amount (0.3%) synthesis of DNA occurs to fill the gap. With the help of another enzyme known ***ligase*** fusion of chromosomal segments with that of opposite site takes place (Stern and Hotta, 1969). The crossing of two chromatids is known as ***chiasma*** (Greek- chiasma means cross) formation. Thus in short crossing over includes the breaking of chromatids, their transposition and fusion.

Chaisma frequency or percentage of crossing over- In one tetravalent the crossing over may take place at several points and hence may result in the formation of several chiasmata. The number of chiasmata depends on the length of the chromosomes because the longer the chromosomes the greater the number of chiasmata. In each species each chromosome has a characteristic or fixed number of chiasmata. The frequency by which a chaismata occurs between any two genetic loci has also shown a characteristic probability. The closely linked two genes have lesser chances for a chiasma formation between them while when two genes situated more apart from each other in a chromosome, the greater the opportunity for a chiasma to occur between them.

4) Terminalization- After the process of crossing over, the non- sister chromatids start to repel each other because the force of synapsis attraction between them decreases. Desynapsis begins during diplotene stage hence synaptical complex dissolves and resulted into two homologous chromosomes in a bivalent are pulled away from each other. After that during ***diakinesis*** chromosomes detach from the nuclear envelope and each bivalent is clearly visible to contain four chromatids with each pair of sister chromatids linked at their centromeres. The chromatids then separate progressively from the centromere towards the chiasma and chiasma automatically itself moves in zipper fashion towards the end of the tetravalent. This movement of chiasma is known as *terminalization.* The terminalization results into complete separation of homologous chromosomes.

Kinds of crossing over- On the basis of number of chiasma formation following types of crossing over have been reported

i) Single crossing over- When the chiasma occurs only at one point of chromosome pair this is known as single crossing over. The single crossing over produces two “Cross Overs” chromatids and two “Non- Cross Over” chromatids.

ii) Double crossing over- When chiasmata occur at two points on the same chromosomes the phenomenon is known as double crossing over. In this case the formation of each chiasma is independent of the other and resulted into four possible classes of recombination. In the double crossing over following two types of chiasma may occur:

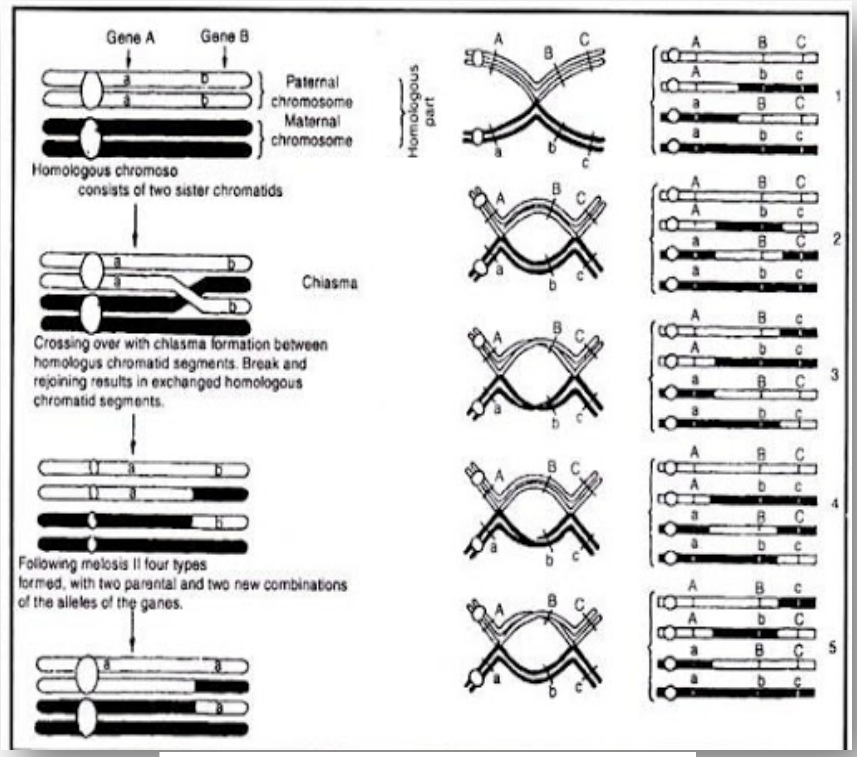

Fig. 10.5 Different types of crossing over

a) Complementary chiasma- When both the chromatids taking part in the second chiasma are different from those chromatids involved in the first chiasma. It produces four single cross overs and no non-cross over.

b) Reciprocal chiasma- When the two chromatids are involved in the second chaisma as in the first. Thus the second chiasma restore the order or sequence which was changed by the first chiasma and this resulted into two non- cross over chromatids.

iii) Multiple crossing over- When crossing over takes place at more than two places in the same chromosomal pair then this is known as multiple crossing over. The multiple crossing over occurs rarely.

Theories about the mechanism of crossing over- There are a few important theories about the mechanism of crossing over produced by different geneticists from time to time, and some important ones are as following:

1. Duplication Theory- John Belling (1928) proposed this theory while studying meiosis in some plant species. According to him crossing over might occur during duplication of homologous chromosomes and might brought about between newly synthesized genes due to novel attachment. He visualized genes as beads (later described as chromosomes) which are connected with each other by non- gene linking elements in the inter chromomeric regions.

Initially the chromosomes are duplicated during duplication of chromosomes and the newly formed chromosomes remain tightly juxtaposed to old ones. When inter chromomeric regions are synthesized to join these new genes or chromosomes they may switch from a newly synthesized chromosome on the homologous chromosomes to an adjacent chromosome of other homologous. This results in the formation of cross over in new sets of chromatids or recombination.

2. Copy-Choice Theory or Switch Model Theory- Laderberg (1955) proposed a new definition of Belling's hypothesis to explain recombination in microorganisms. This modified version of original one is called copy- choice mechanism of recombination or crossing over. According to this model a daughter chromosome is formed by alternate use of recipient and donor chromosome material as a kind of model or template. The daughter chromosome is then like the recipient chromosome except for portions "Copied" from the donor chromosome segment. In brief, the theory assumes that the crossing over is the direct result of the new chromatids copying partly from one strand and partly from other homologous strand. At the end of the process the recipient cell would contain three type segments:

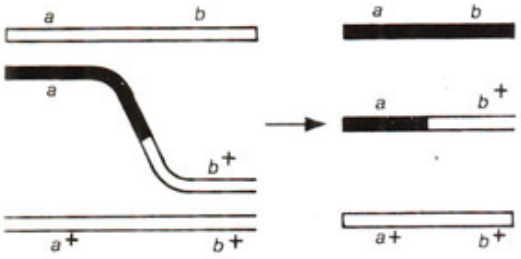

Fig.10.6 Copy- choice model for the mechanism of crossing over

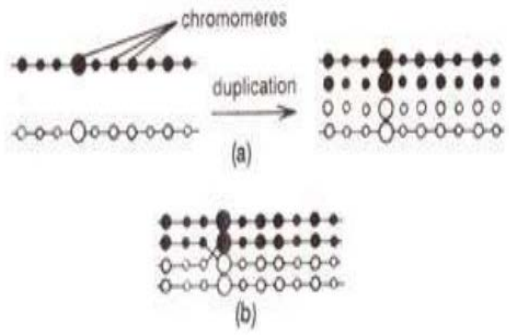

Fig. 10.7 Three steps of Belling's duplication theory

i) Original donor segment

ii) The originally donor recipient whole segment and

iii) A "hybrid" daughter chromosome.

According to Burns (1969) the donor segment is lost in succeeding divisions of recipient cell. The copy- choice model to explain the mechanism of recombination has been criticized for the following two reasons:

a) Based on experimental evidences it is suggested that DNA replication occurs in a semi conservative manner while copy-choice model of DNA replication relies on conservative mode.

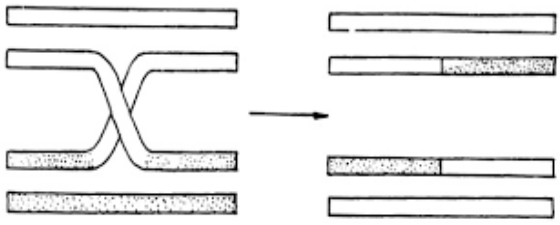

Fig. 10.8 Crossing over according to Copy-choice theory

b) The copy choice model proposed the involvement of only two chromatids in the process of crossing over while according to cytological investigations it is clear that crossing over occurs at four (tetravalent) strand stage.

3. Break and exchange theory- The break and exchange theory is the most accepted theory to explain the process of crossing over. It states that the crossing over takes place due to breakage and reunion of non- sister chromatids. The two segments of parental chromosomes which are present in recombinants arise from physical break in the parental chromosomes with subsequent exchange of broken segments. The break occurs in the non- sister chromatids of tetravalents. This breakage is followed by the exchange of chromosomal material between non- sister chromatids.

Factors affecting crossing over- Experimentally it has been proved that the frequency of crossing over between two genes is not completely dependent on the distance as mentioned earlier but several other physiological and environmental factors can also influence it. Temperature, x- rays and chemical composition of food may be responsible to change the frequency of crossing over. In *Drosophila melanogaster* it may be reduced with the increase of the age of the fly.

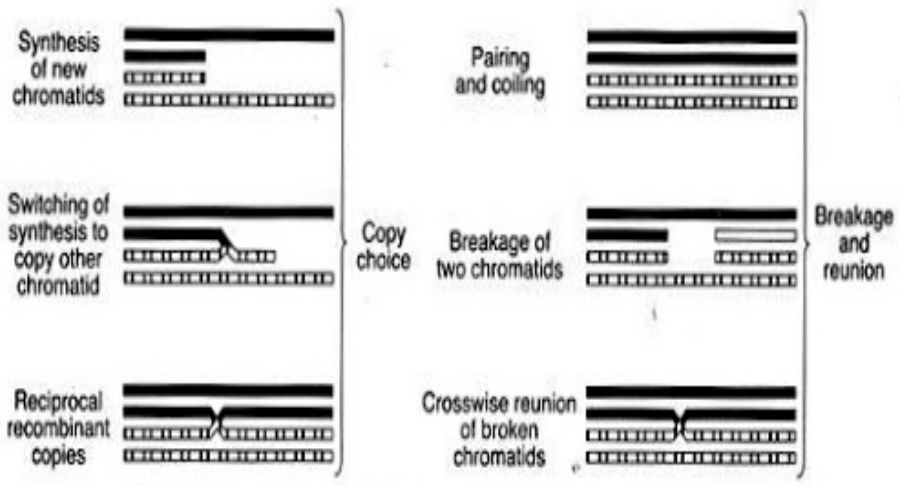

Fig. 10.9 Copy choice and break and exchange theory

Significance of crossing over- Crossing over is a universal phenomenon and occurs in all kinds of organisms e.g. in prokaryotes, eukaryotes, plants, animals, microorganisms, etc. It has so many impacts in genetics and heredity of different organisms as follows:

1. It helps to produce new combinations of traits.
2. Due to crossing over segments of homologous chromosomes are interchanged and hence provide origin of new characters and genetic variations.

3. It plays a very important role in the field of breeding to improve the varieties of plants and animals

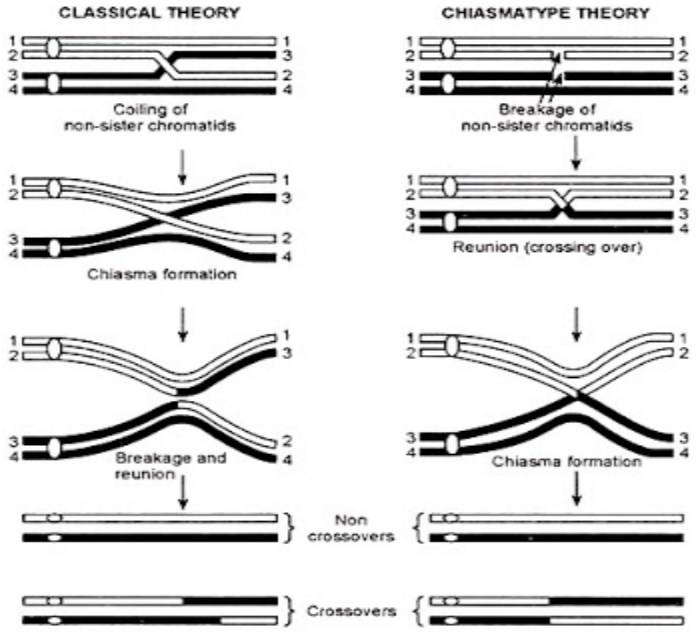

Fig. 10.10 Diagram showing crossing over according to classical and chiasma type theories.

4. Crossing over plays important role in evolution and forms the genetic basis for variation.
5. The gametes produced through meiosis receive a new combination of characters or genes and in each generation individual with new combinations of characters are produced.

10.5 MUTATION

In a species there are different types of variations or differences, they may occur either due to changes in the outer or physical environment or due to change in the inner or hereditary constitution or, due to the combination of both. The variation caused by environmental or outer conditional changes may be restored by changing the environmental conditions (or providing original environment). These changes are not heritable therefore not transferred to the next generations. It means they may not build in the genotype, are temporary and unable to become the bases of evolution. These variations are only able to change the outer morphology of the species/ organism and so also known as phenotypic variations or simply modifications. They are defined as "phenotypic differences between organisms of similar genotype." On the other hand, when variations occur inside in the genes and are related to environmental changes, therefore are irreversible, permanent and heritable. These changes occur in the genome of the individual and may be caused by crossing over or recombination and mutation. Recombination process create genetic diversity at the level of genes that reflects differences in the DNA sequences of different organisms so it usually causes no remarkable variation because it merely redistributes existing genetic material among different individuals. However, mutations are referred as "*sudden*

changes in genotype and developing qualitative and quantitative alterations in the genetic material itself".

Geneticists often distinguish between two kinds of mutations; chromosomal and point mutation. The chromosomal mutations are known as *chromosomal aberration* the later causes alteration or changes in the amount (number) or position of genetic material. Point mutations bring permanent changes within a gene or cistron of the DNA molecule. Here in this chapter we discuss about point mutations.

Historical background- Seth Wright (1791) for the first time reported point mutation when he noticed a lamb with exceptionally short legs in his flock of sheep in Massachusetts, USA. He produced a flock of sheep having short legs by employing artificial breeding technique visualizing the economic significance of short legged sheep. Later this short legged sheep was named as "***Ancon Sheep"*** (sheep with long body and short legs whose front legs are bent). The trait of short legs was found to be a result from a recessive mutation and all the short legged individuals were homozygous recessive.

The term "***Mutation"*** was first used by Hugo de Vries (1901) when he observed it in *Oenothera lamarkiana*. He used word mutation to describe heritable phenotypic changes of evening primrose (*Oenothera lamarkinana*).

Occurrence- In nature, mutation occurs frequently and have been reported in many organisms e.g., *Drosophila*, mice, rats, rabbits, rodents and man. In *Drosophila* mutation studies proves showed many differences in body and eye color and wing shapes viz. black and yellow body, white and pink eye, normal and vestigial wing shapes, etc.

Similarly in rodents coat colors viz., black, brown and white are due to mutations. Even in human beings mutation causes variations in eye color, hair color, skin pigmentation and several other somatic malformations and disorders.

10.5.1 Kinds of Mutation- Geneticist have no clear idea about the possible kinds of mutations. They have been classified variously according to different bases/ ideas as follows:

1. Classification of mutation according to type of cell- according to their presence in germinal (germ cells) and body (somatic) cells following types of mutations have been classified-

a) Somatic mutation- The mutation occuring in body (somatic) or non- reproductive cells are known as somatic mutation. The somatic mutation have no genetic and evolutionary significance and cannot be inherited by the offspring of the parent organism of the mutate cell with the exception of embryo development. The animal body may constitute a large number of body cells and the body may be a mosaic of different type of cells. Somatic mutations have often been related with cancerous or malignant growth. Hugo de Vries have been reported somatic mutation in *Oenothera lamarkiana* and in several other organisms including man. In man somatic mutation causes several fatal diseases such as ***paroxysomal nocturnal, hemoglobinura, circumscribed neurofibroma, unilateral retinoblastoma and heterochromia of iris.*** Mostly only

a single cell and its daughter cells are involved. If, a somatic mutation occurs early during its embryonic stage it causes cancerous growth.

b) Gametic mutation- The mutations occurring in germ cells or gametic cells (e.g., egg and sperm) are called gametic mutations. Such mutations are of great genetic significance and are heritable. These mutations provide raw material for the natural selection of organisms.

2. Classification of mutation according to the size and quality- On the basis of size mutation is categorized as follows-

A. Point mutation- A point mutation is a genetic mutation when heritable changes occur in a very small segment of DNA molecule or a nucleotide or nucleotide pair. The point mutation may occur due to changes in the DNA or RNA at nucleotide level.

i) Deletion mutation- The point mutation caused due to deletion or missing of a single nucleotide pair in a triplet codon of a gene or cistron is called deletion mutation. It is frequently reported in some bacteriophages (e.g. Phage T^4). Diseases that can be caused by deletion mutation include DiGeorge syndrome, Cystic fibrosis, Turner syndrome, etc.

ii) Addition or Insertion mutation- The mutation occuring due to addition of one or more extra nucleotides to a gene or cistron are called addition/ insertion mutation. It can be artificially induced by certain chemical substances or mutagens i.e. acridine dye and proflavins.

Both of these point mutations i.e. deletion and insertion alter the code words and cause the changes in the rest of the messages, since after the deletion or insertion of the nucleotide the rest of the reading frame will be modified. This may lead or result to the death of the cell as this can result in the production of an inactive protein. Huntington's disease and the fragile X syndrome are example of insertion where in trinucleotide repeats are inserted into DNA sequence leading to these diseases. Deletions are mutations in which a section of DNA is lost or deleted.

3. Substitution mutation- In this type of point mutation a nucleotide of a triplet is replaced by another nucleotide. This type of mutation is called substitution mutation and it affects only a particular triplet codon. Such triplet codon which have altered/ changed code word may result in the production of a protein with a single amino acid substitution because it may designate a different amino acid due to changed code word. The substitution mutations are of great genetical significance as it may alter the phenotype of an organism variously. Thus we can say that substitution is a mutation where one base substitutes for another base (i.e. a change in a" single chemical letter "such as switching an A to G. Such a substitution could change a cistron to one that encodes a different amino acid and cause a small change in the protein product. These mutations may be classified into following sub types:

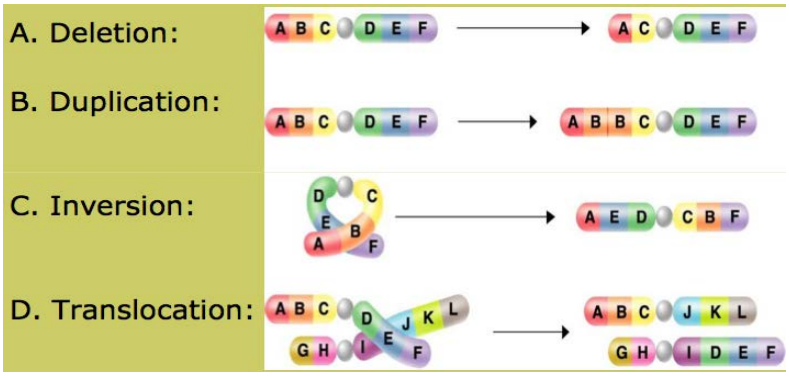

Fig. 10.11 Diagram showing different type of mutations.

i**) Transition-** Transition mutation is that mutation in which a purine nucleotide changes to another purine (A - G) or a pyrimidine nucleotide to another pyrimidine (C -T). This type of mutation is called transition mutation. Transition can be caused by tautomerization, oxidative and deamination. These cause drastic changes in reading frame and protein product or enzyme or phenotypic expression.

Tautomerization- Normally in a DNA molecule the purine- adenine (A) is linked to the pyrimidine- thymine (T), by two hydrogen bonds, while the purine- guanine (G) is linked to the pyrimidine- cytosine (C) by three hydrogen bonds- (A=T, G=C) these are the common configuration but beside this each DNA base may have some altered uncommon molecular configurations also as shown in Fgure-10.12. These uncommon forms of DNA are rare states or tautomers and are generated by single proton shifts. When the amino (NH_2) form of adenine changed to an imino (NH) form is believed to be the cause of a tautomeric shift. Similarly, a tautomer shift may occur in Thymine changing it from the keto (C=O) form to the rare enol (COH) form. Basically when a bond occurs in its tautomer or rare state, it cannot be linked to its normal partner.

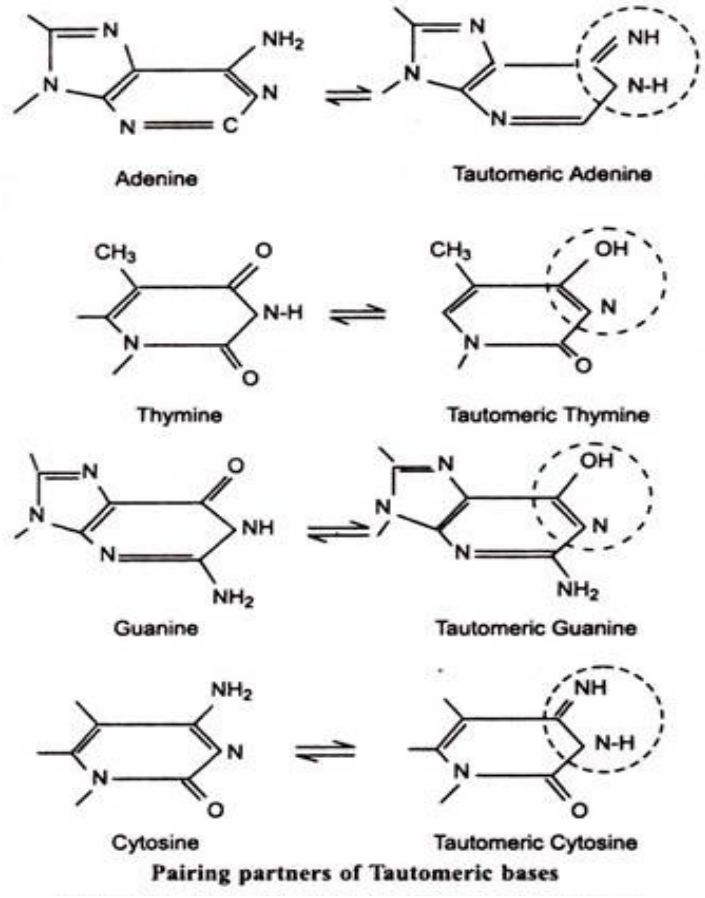

Fig. 10.12 Uncommon forms of tautomeric DNA bases.

However, a purine- thymine and its rare state form a bond with cytosine besides thymine, provided the cytosine in its normal state.

Watson and Crick in 1953 proposed a hypothesis that the bases occur in their rare states provides a mechanism for mutation during DNA replication. For example if in an old chain of DNA adenine is in its rare state at that moment the complementary new chain reaches it and cytosine can pair with it (adenine) beside thymine and be added to the growing end of new chain. The result of this type pairing is the formation of a DNA molecule that contains an exceptional base pair. However, this situation is unstable and at the next replication adenine is expected to return to its common state and to pair with thymine.

Here cytosine introduced into the complementary strand due to tautomeric shift in adenine would than pair with guanine, Thus these would be forming two kind of DNA molecules; i) that is identical to the original DNA and, ii) that has a base pair substitution of G-C for A-T. This DNA molecule which was transitionally substituted has altered/ changed coding at a point and results in recognizable mutation. Such mutations formed during DNA replication are called Copy error mutations. For instance, thymine in its ionized state can pair with guanine (T-G), if the guanine is in its common form.

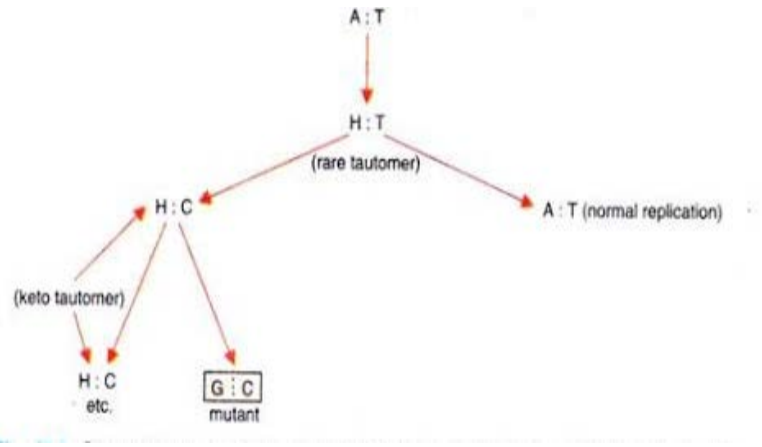

Fig. 10.13 Conversion of A:T pair into G:C pair due to keto tautomerization of adenine.

Similarly, guanine in its ionized form can pair with thymine in its common form. From any such unstable base pair, a transition will result. Experimental evidences for tautomerization-substitution or transition mutations have shown by applying various chemical mutagens by following methods-

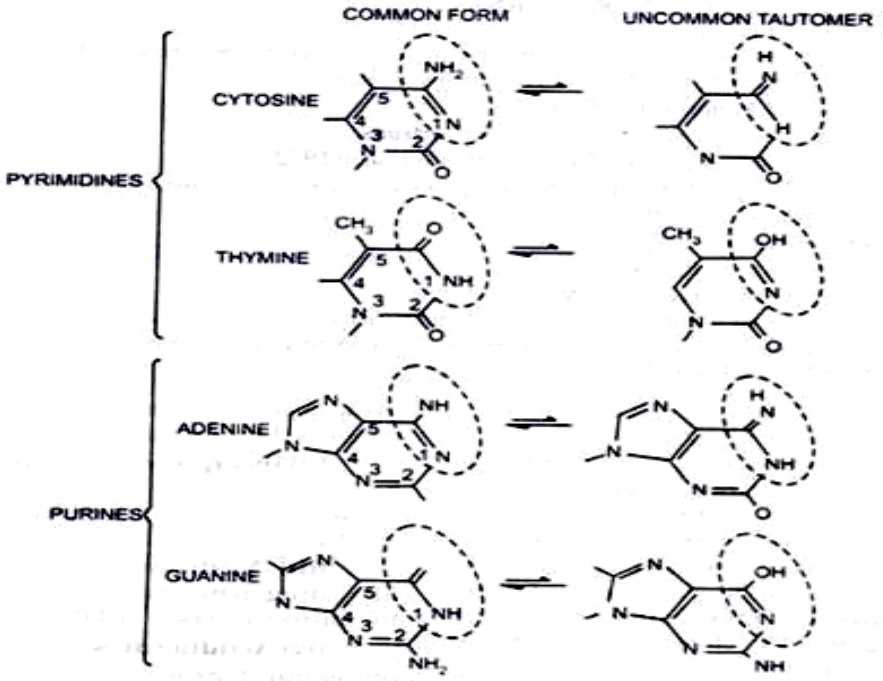

Fig.10.14 Pairing qualities of rare tautomeres of four DNA bases

Deamination- Some chemical substances for example, nitrous acid cause transitional mutation due to oxidative deamination of DNA bases. In oxidative deamination process the amino group or NH_2 of DNA base is replaced by hydroxyl group or OH group by the chemical mutagens. Due to this the adenine is deaminated into hypoxanthine by the action of nitrous acid.

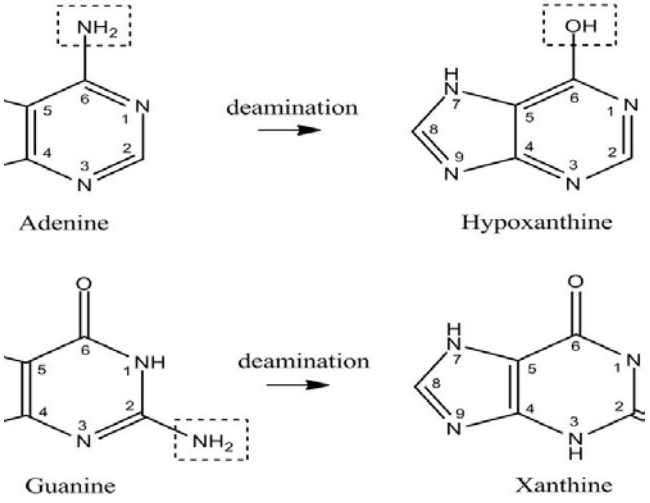

Fig 10.15 Deamination Reaction

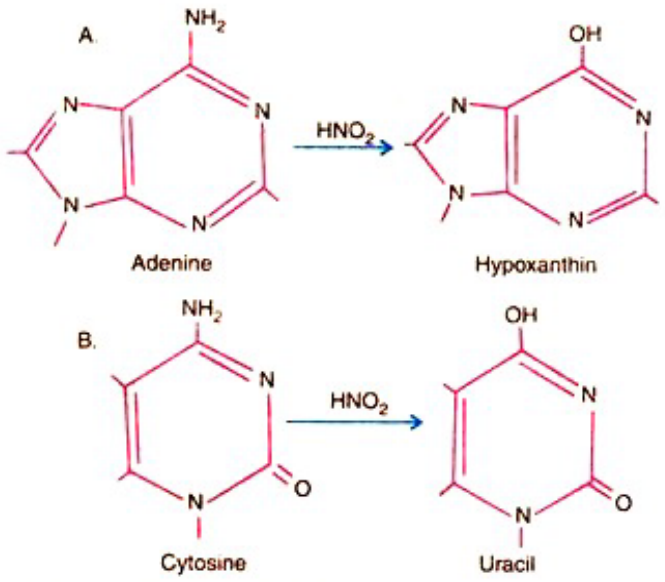

Fig. 10.16 Deamination of adenine into hypoxanthine (A) and cytosine into uracil (B) by nitrous oxide.

The hypoxanthine (HX) is converted into more or common keto- tautomer by tautomeric shift which pairs with cytosine. The A:T pair, thus can be converted to G:C pair. Likewise this, due to deamination cytosine converts into uracil, which has pairing properties similar to thymine and in such case G: C pair would be changed into A: T pair.

Base analogue- Certain chemical substances have molecular structure similar to the usual DNA bases if they are available. They are known as base analogues and may be incorporated into a replicating DNA strand. For example 5- bromouracil (5BU) or its nucleotide 5-bromodeoxyuridine (5- BU) or its nucleosides 5- bromodeoxyuridine (5- BURD) in its usual keto form in a structural analogue of thymine (5 methyl uracil), will substitute for thymine. Due to this an A- T pair become and remains A- BU. Since 5-bromouracil can pair with either adenine or guanine, it also affects base pairing during DNA replication which leads to mutation. An analogue of adenine, 2, amino purine, also causes mutation in a similar way. It can pair with either T or C, 5- bromouracil is used to treat neoplasm in the form of its nucleoside. 5- Bromo 2-synthesis and metabolism. 6- Mercaptopurine is used to treat acute leukemia.

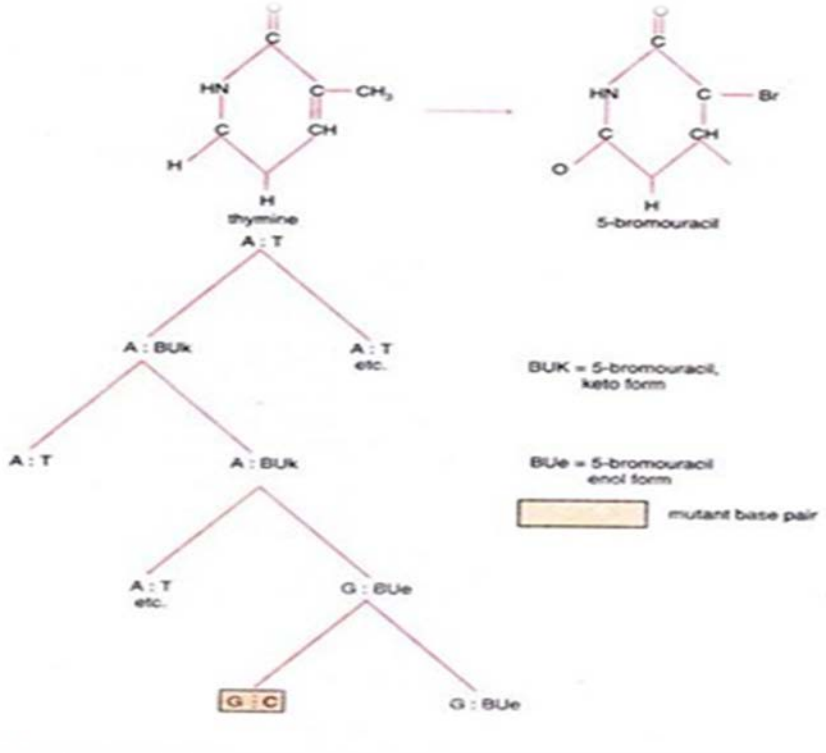

Fig.10.17 Conversion of A:T base pair and G:C base pair due to keto and enol forms of bromouracil

Base analogue mutagens are chemicals that mimic bases to such an extent that they can be incorporated into DNA in place of one of the normal bases but by doing so it leads to mutation. 2- Amino purine- (2- AP) is also a base analogue which is relatively undifferentiated purine that apparently can with cytosine with thymine. It is thought that 2- AP acts by "switching" pyrimidine.

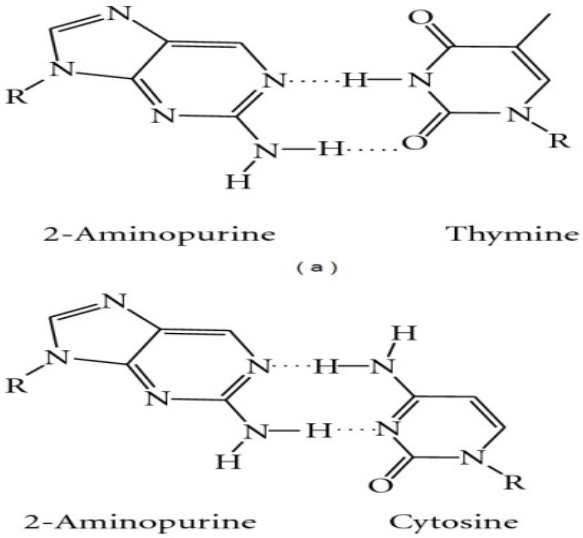

Fig. 10.18- Aminopurine a base analogue of Thymine and Cytosine

Transversion- The substitution mutation may also occur when a purine is replaced with a pyrimidine or when a pyrimidine is replaced with a purine. This type of substitution mutation is called transversion mutation. This was first reported by Freese (1959). As a result of transversions the mutated position in the gene may vary for example, it may have an adenine where it had a thymine or cytosine earlier.

Transversions are interchange of purine for pyrimidine bases which therefore, involve exchange between one - ring and two- ring structures. Genetically it is extremely difficult to recognize or identify transverse mutation and still there is poor information about the mechanism of its induction, identification and characterization of transverse ion mutations. Analysis of amino acid substitutions in purines is the only way to recognize this type of mutation.

It is revealed that low pH value and high temperature are effectively responsible for the loss of purine bases or depurination.

Gross mutation- When changes involve the entire gene or more than one nucleotide pair, such type of mutation is called gross mutations. The main cause behind such mutation may be the rearrangement of gene within the genome and may be of following three types:

i) When rearrangement of genes may occur within a gene. Two mutations occur within the same functional gene whether they are present in Cis or trans position can produce different effects.
ii) When rearrangement of genes may occur in number of genes per chromosome. If on the homologous chromosomes, the number of gene replicas is non- equivalent, they may cause different type of phenotypic effects over the organism.
iii) When mutation occurs due to movement of a gene locus, new type of phenotype may be created, especially when the gene is relocated near heterochromatin. The movement of gene loci may take place by the following methods.

a) **Translocation -** When movement of gene may take place to non- homologous-chromosomes it is known as translocation.

b) Inversion When movement of genes occurs within the same chromosomes it is called inversion. In this case rejoining of chromosomes may occur within a chromosome, a chromosome segment between the two break points becomes involved and thus called an inversion.

3. Classification of mutation according to the origin. On the basis of mode of origin following two types of mutations have been recognized:

A. Spontaneous or natural mutation These mutations occur suddenly in the nature and are also known as back ground or spontaneous mutations. Such mutations have been reported in many organisms i.e., microorganisms, bacteria, viruses, bread moulds, *Drosophila*, maize, man, mice, *Oenothera,* etc. The rate of such mutations is very slow.

B. Induced mutation Besides naturally or spontaneously occurring mutation sometimes mutations can be induced artificially in the living organisms by exposing them to abnormal environmental conditions using different types of radiation, chemicals and certain physical conditions (i.e., temperature). The substances or segment which induces artificial mutations are called mutagens or mutagenic agents.

Mutagenic agents: Those agents responsible for the changes in genetic material usually DNA of an organism. They may be either physical or chemical agents and thus increase the frequency of mutations above the natural background. The mutagenic agents are of following types.

a. Physical Mutagens
b. Chemical Mutagens
c. Biological Mutagens

a.) Physical Mutagens Radiations which are important in mutagenesis are of two types-
i) Ionizing Radiations and ii) Non- ionizing Radiations

i) Ionizing Radiations- Those radiations which possess ion forms such as x- rays, α, β, γ rays, electrons, protons, neutrons and other fast moving particles are categorized under this category.

Ionizing radiations as mutagenic agents- There is a little knowledge about the mechanism by which ionizing radiation cause mutation. As clear from studies that any matter composed of atoms and a single atom constitutes a positively charged atomic nucleus having neutrons in its outer orbit. The normal atoms are electrically neutral because of the balanced charges of the atomic particles. When ionizing radiation passes through matter, they dissipate their energy in part through the ejection of electrons from the outer shell/ orbit of atom and the loss of these balancing negatively charged particles or electrons leave atom which are now positively charged and does not remain neutral. These positively charged atoms are called ions. Now the ejected electrons of atoms move at higher speed and push other electrons free from their respective atoms and dissipate their energy and become attach to other atoms and convert the ion into negatively charged ions. To achieve their stable or neutral charge ions undergo many chemical reactions and during these chemical reactions it is thought that ionizing radiation causes mutation.

These ionizing radiations able to break the poly sugar phosphate backbone of DNA and thus cause chromosomal mutations i.e. deletion, addition, break, inversion and translocation. Due to ionizing radiation during breakage of DNA molecule the active role of oxygen is predicted because oxygen is important to the formation of H_2O_2 and H_2O in irradiated water and their products may induce break in DNA molecule.

ii) Non- Ionizing radiations as Mutagens-

This category includes the UV radiations which may cause mutation. About 2600 A^0 refers as the most effective wavelength of ultra violet light to induce mutations. This is a wavelength that is best absorbed by DNA and a wavelength at which protein absorb little energy. When a substance

absorbs sufficient energy from the ultra violet light some of their electrons are raised to higher energy levels. This state is known as excitation. The excited molecule becomes reactive and mutated named as photoproducts.

Demerization- The ultraviolet radiation affected DNA molecule. Among them one of the effect is the formation of chemical bonds between two adjacent pyrimidine molecules of a polynucleotide and especially between adjacent thymine residues. The two thymine residues associate or simply dimerize and form a dimer. Due to this, that they can no longer form hydrogen bonds with the opposing purines as their position in the DNA helix becomes so displaced and thus the regularity of the helix becomes distorted. Thus dimerization interferes with the proper base pairing of thymine with adenine, and may result in thymine's pairing with guanine and produce a T - A to C - G transition.

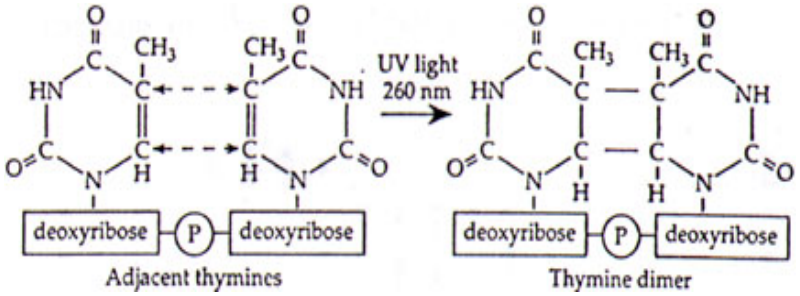

Fig.10.19 Formation of a dimer of thymine.

iii) Temperature as mutagen- Temperature also plays role of mutagen. Temperature influences the rate of all chemical reactions. Thus it is not surprising that temperature can be mutagenic. It is reported that increase in temperature increases the rate of mutation. For example, an increase of 10^0 C temperature increases two to three fold of mutation rate. Temperature probably affects the thermal stability of DNA and the rate of reaction of other substances with DNA.

b. Chemical mutagens Besides temperature, many chemical substances are also responsible to increase mutations in genes. The ability of chemical substances to induce mutation was first of all demonstrated by Auerbach and Robson in 1947 using mustard gas and related compounds as the nitrogen and sulphur mustards, mustard oil and chloroacetone in experiments with male *Drosophila melanogaster*. Later many non- toxic ordinary chemical compounds have been found to be mutagenic in certain specific situations. Any chemical substance that involves mutation effecting the chemical environment of chromosomes is likely to influence at least indirectly as the ability of DNA to replicate without error and the stability of DNA. A chemical mutation can cause only when it enters the nucleus of the cell. The chemical substances affect the chromosomal DNA by following two ways:

i) Direct gene changes and
ii) Copy error.

Direct gene change Certain chemical mutagens directly affect DNA thus it is known as direct gene change. They affect the constituents of DNA only when DNA is not replicating. For example- Nitrous acid converts adenine into hypoxanthine and cytosine to uracil by deamination. Nitrogen mustard, formaldehyde, epoxides, dimethyl and diethyl sulphate and ethyl methanosulphonate (MMS and EMS) and Nitrosoguanine (NG) also have direct mutagenic effect on the DNA synthesis like nitrous acid.

Copy error Certain chemical compounds are called base analogues which are discussed earlier in this chapter. Base analogues are molecules which have a very similar structure to one of the four nitrogenous bases which are used in DNA (adenine, guanine, cytosine and thymine). They form a similar structure to one of the DNA nucleotides and thus can be used to form the new DNA strand in semi conservative replication (e.g. 5- bromouracil, 2- aminopurine, etc.). They closely resemble with certain DNA bases and are, therefore, acts like mutagens. When DNA replication takes place they are incorporated by DNA in place of the normal DNA bases. Besides this certain other base analogues have also mutagenic effects such as caffeine, (presents in coffee and soft drinks), phenols, acridine (proflavins), carcinogens, triazine, etc. Certain inorganic substances manganese chloride causes mutation in many organisms because these compounds bind calcium and thus interfere with the integrity of the chromosome structure.

4. Classification of mutations according to the origin Based on their mode of direction following two types of mutations have been reported:

a) Forward mutation When mutation create a change in a organism from wild (natural) type to abnormal phenotype then that type of mutations are known as forward mutations. In nature most of the mutations are forward type.

b) Back or reverse mutations When an abnormal phenotype changes into wild type, this type of mutation is known as back or reverse mutations. They are often corrected mechanism. They may be of following types-

i) Single Site Mutation

ii) Mutation Suppressor

i) Single site mutation Some reverse mutations change only one (single) nucleotide in the gene and thus are called single site mutations, e. g. due to forward mutation the adenine is changed into guanine and backward mutation changed guanine into adenine

Forward Mutation Reverse Mutation

Adenine----------Guanine----------Adenine

ii) Mutation Suppressor As cleared from the name that when a mutation occurs at a different site from that site where primary mutation already occurred and that mutated (secondary) gene reversed that effect of primarily mutated gene then such (secondary) mutations are called mutation suppressors. They may be of following four types.

a) Extragenic Suppressor When mutation occurs in a different gene from that of the mutated gene then that is known as extragenic suppressor mutation. For instance in *E. coli* rec A is a gene mutation suppressor gene (rec- recombination gene) which is necessary for recombination and is found to repair ultraviolet induced Thymine dimer of a gene caused by a process- photo replication recombinational repair.

b) Intragenic Suppressor The intragenic suppressor mutation occurs within the same gene but in a different nucleotide and hence shifts the frame back into register.

c) Photoreactivation In this type of reverse mutation reversal of ultraviolet induced thymine dimer takes place in the presence of visible light by specific enzyme. A particular enzyme is selectively bond to the bacterial DNA during ultra violet radiation. During photo reactivation the enzyme is activated by visible light and cleave pyrimidine or purine dimers into their monomers and restores their original form. In general, photo reactivation is a light induced enzymatic cleavage of a thymine dimer to yield two thymine monomers. Phototype is also active against cytosine - thymine dimers, which are also forwarded by UV irradiation but much less frequently.

d) Dark reactivation or Excision repair In the absence of light (or in dark) the reverse mutation may also occur in an ultra violet induced mutation. Howard Flanders and Boyce (1964) put forth hypothesis regarding the presence of Excision repair. According to them:

i) An enzyme possibly endonuclease make a cut in the polynucleotide strand on either side of the dimer which may be formed due to ultraviolet radiation and excises a short single strand segment of DNA.

ii) Another enzyme possibly endonucleases widens the gap produced by the action of the endonucleases.

iii) The final gap (made by endonucleases enzyme) closed by enzymatic rejoining process, as yet not clear.

5. Classification of mutation according to phenotypic magnitude On the basis of their phenotypic effects following types of mutations may occur.

i.) Dominant mutation The mutations expresses the dominant phenotypic expressions are called dominant mutations. For example in man aniridia (absence of the iris of eyes) a mutation disease occurs due to a dominant mutant gene.

ii.) Recessive mutation In nature most type of mutations are recessive type. They are not expressed phenotypically (morphologically) immediately and their effect even can be seen only after one or more generations when the mutant genes is able to recombine with another similar recessive gene.

iii.) Isoalleles Sometimes some mutations change the phenotype of an organisms so slightly that they cannot be detected. For this special techniques are applied. They produce identical phenotype in homozygous or heterozygous combinations.

iv.) Lethal mutation Some mutations cause the death of the organism and hence are called lethal mutation.

6. Classification of mutation according to the type of chromosomes Based on the types of chromosomes the mutations may be of following two types:

i.) Autosomal Mutation- When mutation occurs in autosomal chromosomes (non- germinal) then this is called as autosomal mutation.

ii.) Sex Chromosomal Mutation- When mutation occurs in sex or germ (egg and ovum) chromosomes, this is called sex chromosomal mutation.

Mutation rate/ mutation frequency The rate or frequency with which genes mutate spontaneously in nature is called mutation rate. Mostly the genes remain stable and mutation takes place rarely. The great majority of genes have mutation rate of 1×10^{-4} to 1×10^{-6}, one gamete in 1,00, 000 to one gamete in million would contain a mutation at a given locus. The mutation rate is affected by various external and internal factors as follows-

a. Environmental control of mutation rate
b. Viral control of mutation rate and
c. Genetic control of mutation rate

Viral Control of Mutation Rate- Sprage (1963) proves that virus affect the mutability of host's genes. Viruses increase the mutation rate in *Drosophila melanogaster* (Baumiliar, 1967).

Genetic Control of Mutation Rate- The studies showed that mutation rate is under control e. g. certain genes called mutator genes may increase the mutation rate in *Drosophila*, *E. coli* and maize. However, certain suppressor genes may decrease the rate of mutation.

Significance of mutation Mutation is the change in the DNA at a particular locus in an organism. Mutation is the ultimate source of new alleles in plants, animals, microorganisms, human beings, etc. Mutation plays an important role in evolution. In majority of cases mutations are deleterious to the organisms and have shown least effects on any populations because of the action of natural selections. Comparatively to wild or natural type individuals mutant type individuals have shown less survival. The number of expected mutant types are appear less frequently than expected even under optimal environmental conditions.

10.6 SUMMARY

In this chapter we discussed about linkage, crossing over and mutation. It is evident from studies that all these chapters are interrelated with one another. Genetic linkage can be defined as the tendency for alleles closely associated on the same and to be transmitted as an intact unit through meiosis. Crossing over is a basic concept of genetics and cell biology and often called recombination. It occurs during meiosis. Crossing over is of a great biological significance and a big factor in the genetic diversity of living organisms.

Mutations are the sudden changes in the genetic makeup caused either due to external changes or due to internal changes in the genes. In biology, a mutation is the alteration of the nucleotide sequence of the genome of an organism.

10.7 GLOSSARY

Centromere- It is a specialized DNA sequence of a chromosome that links a pair of sister chromatids (a dyad).

Codon- Codon is the name given to a stretch of the three nucleotides. A codon is a trinucleotide sequence of DNA or RNA that corresponds to a specific amino acid.

Coupling- Alleles coming from the same parents tend to enter the same gamete and are together in most cases.

Crossing Over- The exchange of genes between homozygous chromosomes resulting in a mixture of parental characteristics in offspring.

Deletion Mutation - Any number of nucleotides can be deleted from a single base to an entire piece of chromosome.

DNA Mutation- There is three types of DNA mutations, base substitution, deletion and insertions.

Genome- The complete set of genes in an organism or the total genetic content in one set of chromosome.

Genotype- The genetic makeup of an individual often times in reference to a particular gene.

Homozygous- Having identical alleles for a given gene.

Heterozygous- Having two different alleles for given genes, both alleles at corresponding loci are dissimilar.

Insertion- Addition of one or more nucleotide base pairs into a DNA sequence.

Lethal mutation- Lethal mutations lead to the death of the individual.

Linkage Group- A pair or set of genes on a chromosome that tend to be inherited together.

Mutation- Accidental, random changes in a DNA sequence caused by environmental factors and recombinations.

Mutant Allele- Any form of that allele other than the wild type is known as a mutant allele.

Point Mutation- A mutation affecting only one or very few nucleotides in a gene sequence.

Prophase^{Ist}- Meiosis occurs in two stages, meiosis Ist and Meiosis IInd. In meiosis Ist also known as reduction division, there is the series of events that results in the formation of two haploid daughter cells.

Recessive- An allele that produces its characteristic phenotype only when the paired alleles are the same, will be masked. It is dominant allele present in F_1 generation.

Recombination- Recombination is a process by which pieces of DNA are broken and recombined to produce new constitution of alleles.

Repulsion- Genes coming from different gametes and to be inherited separately or independently in most cases is known as repulsion.

Single base pair-A point mutation or substitution is a genetic mutation where a single nucleotide base is changed, inserted or deleted from a sequence of DNA.

Sister Chromatid- The two copies of each chromosome often remain stuck together until they are separated with one copy going to each daughter cell. While stuck together these two copies are called" sister chromatids".

Substitution Mutation- It is a mutation that exchanges one base for another base.

Wild type- (WT) Refers to the phenotype of the typical form of a species as it occurs in nature.

10.8 SELF ASSESSMENT QUESTIONS

Q. Define linkage or what do you mean by genetic linkage?
Q. Define linked genes.
Q. What are linkage groups?
Q. Who discovered linkage?
Q. What is Crossing over? Describe different steps of crossing over?
Q. Define coupling and repulsion theory of Bateson and Punnett.
Q. What do you mean by synaptical complex? Describe its structural composition.
Q. Define complete and incomplete crossing over and differences between them.
Q. Define mutation and its types.
Q. Describe different types of mutagenic agents.
Q. Define Point mutation.
Q. Define Gross mutation and classify it briefly.
Q. Write short notes on following-
i) Transition, ii) Tautomerization, iii) Deamination, iv) Base analogue
Q. Write short notes on following-
i) Complete linkage, ii) Incomplete linkage, iii) Synaptonemal complex, iv) Chiasma
Q. Write short notes on following-
i) Belling's hypothesis, ii) Deletion mutation, iii) Transversion mutation, iv) Physical mutagens

10.9 REFERENCES

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10.11 TERMINAL QUESTIONS

Q. Describe linkage and independent assortment? Explain with suitable examples.
Q. Who gave the term linkage?
Q. Define Sutton's view on linkage.
Q. Describe briefly incomplete linkage in maize.
Q. Define the chromosome theory of linkage.
Q. Define Cis and Trans theory.
Q. Define crossing over and its significance.
Q. Describe the functions of synaptical complex.
Q. Write short notes on any three of the following:
i) Crossing over, ii) Duplication of chromosomes, iii) Mutagenic agents, iv) Chemical Mutagens
Q. Write short notes on any three of the following
i) Terminalization, ii) Chiasma frequency, iii) Reciprocal crossing over, iv) Complementary crossing over
Q. Define Copy- choice theory and, Break and exchange theory.
Q. Describe briefly different types of back or reverse mutation.
Q. Give detailed account of classification of mutation according to magnitude of phenotypic effects.

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