APPOLO STUDY CENTRE CHENNAI

APPOLO STUDY CENTRE

GENETICS

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10th book
Unit – 18 -Heredity

Gregor Johann Mendel - Father of Genetics

- Mendel (1822-1884) was an Austrian monk who discovered the basic principles of heredity through his experiments. His experiments are the foundation for modern genetics. He was born in 1822 to a family of farmers in Silesian of Czechoslovakia. After finishing his high school at the age of 18, he entered the Augustinian Monastery at Brunn as a priest. From there he went to the University of Vienna for training in physics, mathematics and natural science. Mendel returned to the monastery in 1854 and continued to

work as a priest and teach in high school. In his leisure time he started his famous experiments on the garden pea plant. He conducted his experiments in the monastery for about nine years from 1856 to 1865. He had worked on nearly 10000 pea plants of 34 different varieties. Mendel noted that they diff er from one another in many ways.

Mono hybrid Cross – Inheritance of One Gene

- crosses involving inheritance of only one pair of contrasting characters are called monohybrid crosses. For example it is a cross between two forms of a single trait like cross between tall and dwarf plant

Mendel ’s Explanation of Monohybrid Cross:

- Parental generation: Pure breeding tall plant and a purebreeding dwarf plant **F1 generation:** Plant raise from the seeds of pure breeding parental cross in F1 generation were tall and monohybrids.

- **F2 generation:**

  Selfing of th F1 monohybrids resulted in talland dwarf plants respectively in the ratio of 3:1.The actual number of talland dwarf plants obtained by Mendel was 787 tall and 277 dwarf External expression of a particular trait is known as phenotype. So the phenotypic ratio is 3:1.

  - In the F2 generation 3 different types were obtained:
  - Tall Homozygous – TT(Pure) – 1
  - Tall Heterozygous – Tt -2
  - Dwarf Homozygous – tt– 1
  - So the genotypic ratio 1:2:1.A genotype is the genetic expression of an organism.

Mendal ’s Interpretation on Monohybrid cross

- Based on these observations it was confirmed by Mendel that ‘factors’ are passed on from one generation to another, now refered to as genes. Tallness and Dwarfnessare determined by a pair for contrasting factors , tall plant possess a pair of factors (represented by T- taking the first letter of the dominant character) and a plant is dwarf because it possess factors for dwarfness (represented as t- recessive character). These factors occur in

pairs and may be alike as in pure breeding tall plants (TT) and dwarf plants (tt). This is referred to as homozygous. If they are unlike (Tt) they are referred to as heterozygous.

- Two factors making up a pair of contrasting characters are called allelesor allelomorphs. One member of each pair is contributed by one parent

- When two factors for alternative expression of a trait are brought together by fertilization only one expresses itself, (tallness) masking the expression of the other (dwarfness). The character which expresses itself is called dominant condition and that which is masked is called recessive condition.

- The factors are always pure and when gametes are formed, the unit factors segregate so that each gamete gets one of the two alternative factors. It means that factors for tallness(T) and dwarfness(t) are separate entities and in a gamete either T or t is present. When F_1 hybrids are self crossed the two entities separate and then unite independently, forming tall and dwarf plants.

Dihybrid Cross- Inheritance Two Genes and Law of Independent Assortment:

- Dihybrid cross involves the inheritance of two pairs of contrasting characteristics (or contrasting traits) at the same time. The two pairs of contrasting characteristics chosen by Mendel were shape and colour of seeds: round-yellow seeds and wrinkled-green seeds.

- Mendel crossed pea plants having round-yellow seeds with pea plants having wrinkled-green seeds. Mendel made the following observations:

- Mendel first crossed pure breeding pea plants having round-yellow seeds with pure breeding pea plants having wrinkled-green seeds and found that only round-yellow seeds were produced in the first generation (F_1). No wrinkled-green seeds were obtained in the F_1 generation. From this it was concluded that round shape and yellow colour of the seeds were dominant traits over the wrinkled shape and green color of the seeds.

- When the hybrids of F_1 generation pea plants having round-yellow seeds were cross-bred by self pollination, then four types of seeds having

different combinations of shape and color were obtained in second generation or F_2 generation. They were round yellow, round-green, wrinkled yellow and wrinkled-green seeds.

- The ratio of each phenotype (or appearance) of seeds in the F_2 generation is 9:3:3:1. This is known as the Dihybrid ratio.

- From the above results it can be concluded that the factors for each character or trait remain independent and maintain their identity in the gametes. The factors are independent to each other and pass to the offsprings (through gametes).

Results of a Dihybrid Cross:

Mendel got the following results from his dihybrid cross

- **Four Types of Plants:**
  A dihybrid cross produced four types of F2 offsprings in the ratio of 9 with two dominant traits, 3 with one dominant trait and one recessive trait, 3 with another dominant trait and another recessive trait and 1 with two recessive traits.

- **New Combination:**
  Two new combinations of traits with round green and wrinkled yellow had appeared in the dihybrid cross (F2 generation).

Mendel's Laws

- Based on his experiments of monohybrid and dihybrid cross, Mendel proposed three important laws which are now called as Mendel's Laws of Heredity.

Law of Dominance:

- "When two homozygous individuals with one or more sets of contrasting characters are crossed, the characters that appear in the F1 hybrid are dominant and those that do not appear in F1 are recessive characters".

Law of Segregation or Law of purity of gametes:

- "When a pair of contrasting factors or genes or allelomorphs are brought together in a heterozygote or hybrid, the two members of the allelic pair remain together without mixing and when gametes are formed, the two separate out, so that only one enters each gamete."

Law of independent assortment:

- "In case of inheritance of two or more pairs of characters simultaneously, the factors or genes of one pair assort out independently of the other pair."

Down's syndrome

- This condition was first identified by a doctor named Langdon Down in1866.

- It is a genetic condition in which there is an extra copy of chromosome 21 (Trisomy 21). It is associated with mental retardation, delayed development, behavioural problems, weak muscle tone, vision and hearing disability are some of the conditions seen in these children.

Gene or point mutation

- Gene mutation is the changes occurring in nucleotide sequence of a gene. It involves substitution, deletion, insertion or inversion of a single or more than one nitrogenous base. Gene alteration results in abnormal protein formation in an organism.

Sickle cell anaemia is caused by the mutation of a single gene. Alteration in the gene brings a change in the structure of the protein part of haemoglobin molecule. Due to the change in the protein molecule, the red blood cell (RBC) that carries the haemoglobin is sickle shaped.

Unit – 19 -Origin & Evolution of Life

Theories on Origin of Life:

- Many theories have been postulated to explain the origin of life. The views on the origin of life has been putforth as

Special creation:

- This idea embodies that life on Earth is a divine creation and also attributes to supernatural event at a particular time in the past.It also emphasizes that life has not changed ever since its origin.

Spontaneous generation (Abiogenesis):

- According to this theory life originated spontaneously from lifeless matter. It was believed that fishes originated from mud, frogs from moist soil and insects from decaying matter.

Biogenesis:

- It was speculated by Louis Pasteur (1862) that life originates from pre-existing life. He showed that pre-sterilised flasks kept closed airtight, with killed yeast, did not give rise to any life form, while in another flask kept open to air living organisms arose from killed yeast.

Extraterresterial or Cosmic origin:

- Some scientists still believe that life came from outer space. This states that units of life called spores (Panspermia) were transferred to different planets including earth. This is still an idea of some astronomers.

Chemical Evolution of Life:

- This idea was developed by Oparin (1922) and Haldane (1929). They proposed that with the conditions prevailing on earth, life arose by a series of sequential chemical reactions. The first form of life could have come from pre-existing non-living inorganic molecules which gave rise to formation of diverse organic molecules which are transformed into colloid system to

produce life. The modern concept on chemical evolution regarding origin of life was accepted.

Evidences from Morphology and Anatomy

- The comparative study of morphology and anatomy of animals, reveal that they possess common set of characteristics.

Homologous organs:

- The homologous organs are those which have inherited from common ancestors with similar developmental pattern in embryos. The fore limbs of mammals are homologous structures. A human hand, a front leg of a cat, flipper of a whale and a bat's wing look dissimilar and adapted for different functions. Their mode of development and basic structure of bone are similar.

Analogous organs:

- The analogous organs look similar and perform similar functions but they have different originand developmental pattern. The function of the wings of a bat, the wings of a bird and wings of an insect are similar, but their basic structures are different.

Vestigial organs:

- The degenerated and non-functional organsof animals are called vestigial organs. The same organs are found to be well-developed and functional, in some of the related forms. Some of the vestigial organs in man are vermiform appendix, nictitating membrane, caudal vertebra, coccyx etc.

Atavism:

- The reappearance of ancestral characters in some individuals is called atavism. e.g. Presence of rudimentary tail in new born babies, presence of thick hair on the human body.

Evidences from Embryology

- The study of comparative embryology of different animals, supports the concept of evolution. The embryos from fish to mammals are similar in their early stages of development. The differentiation of their special characters appear in the later stages of development.

- **Biogenetic law** or **Recapitulation theory** was given by Ernst Haeckel. According to this theory, Ontogeny recapitulates Phylogeny. The stages of development of the individualanimal repeats the evolutionary history of the entire race of the animal.

Evidences from Palaeontology:

- Palaeontology deals with the study of fossils. Leonardo da Vinci is called the Father of Palaeontology. The study of fossils helps us to understand the line of evolution of many invertebrates and vertebrates. Fossil records show that the evolution has taken a gradual process from simple to complex organisms. The origin of modern birds is supported by the evidences from palaeontology.

Archaeopteryx:

- Archaeopteryx is the oldest known fossil bird. It was an early bird-like form found in the Jurassic period.It is considered to be a connecting link between reptiles and birds. It had wings with feathers, like a bird. It had long tail, clawed digits and conical teeth, like a reptile.

Theories of Evolution

- Life had evolved along with evolution of earth towards the end of 18th century. Evolution is the gradual change occurring in living organisms over a period of time. Formation of new species due to changes in specific characters over several generations as response to natural selection, is called evolution. The natural changes occuring is explained through the theories of evolution as proposed by Lamarck and Darwin.

Lamarckism:

- Jean Baptiste Lamarck (1744-1829) was a French naturalist, well known for his theory of evolution. Lamarck's theory of evolution was published in 'Philosophic Zoologique' in the year 1809. It is popularly known as 'Theory of inheritance of Acquired Characters" or "Use and Disuse theory" or Lamarckism.

Principles of Lamarckism

I. Internal vital force

- Living organisms or their component parts tend to increase in size continuously. This increase in size is due to the inherent ability of the organisms

II. Environment and new needs

- A change in the environment brings about changes in the need of the organisms. In response to the changing environment, the organisms develop certain adaptive characters. The adaptations of the organisms may be in the form of development of new parts of the body.

III. Use and disuse theory

- Lamarck's use and disuse theory states that if an organ is used constantly, the organ develops well and gets strengthened. When an organ is not used for a long time, it gradually degenerates.

- The ancestors of giraffe were provided with short neck and short forelimbs. Due to shortage of grass, they were forced to feed onleaves from trees. The continuous stretching of their neck and forelimbs resulted in the development of long neck and long forelimbs which is an example for constant use of an organ. The degenerated wing of Kiwi is an example for organ of disuse.

IV. Theory of Inheritance of acquired characters

- When there is a change in the environment, the animals respond to the change. They develop adaptive structures. The characters developed by the animals during their life time, in response to the environmental changes are called acquired characters. According to Lamarck, the acquired characters are transmitted to the offspring by the process of inheritance.

Unit - 20 - Breeding & Biotechnology

Mutation Breeding

- Mutation is defined as the sudden heritable change in the nucleotide sequence of DNA in an organism. It is a process by which genetic variations are created which in turn brings about changes in the organism. The organism which undergoes mutation is called a mutant.

- The factors which induce mutations are known as mutagens or mutagenic agents. Mutagens are of two types namely physical mutagens and chemical mutagens.

Physical mutagens

- Radiations like X-rays, α, β and γ-rays, UV rays, temperature etc. which induce mutations are called physical mutagens

Chemical mutagens

- Chemical substances that induce mutations are called chemical mutagens. e.g. Mustard gas and nitrous acid. The utilisation of induced mutation in crop improvement is called mutation breeding.

Hybridization

- Hybridization may be defined as the process of crossing two or more types of plants for bringing their desired characters together into one progeny called hybrid. Hybrid is superior in one or more characters to both parents. Hybridization is the common method of creating genetic variation to get improved varieties.

Hybridization Experiment: Triticale (The first man – made cereal)

- Triticale is the first man- made cereal hybrid. It is obtained by crossing wheat (Triticum durum, 2n = 28) and rye (Secale cereal, 2n = 14). The F1 hybrid is sterile (2n = 21). Then the chromosome number is doubled using colchicine and it becomes a hexaploid Triticale (2n = 42).

- The cycle of crop raising and selection continues till the plants with the desired characters are finally obtained. The development of new varieties is a long-drawn process. Two main aspects of hybridization are to combine the characters of two plants in one plant and to utilize hybrid vigour.

Genetic Engineering

- Genetic engineering is the manipulation and transfer of genes from one organism to another organisms to create a new DNA called as recombinant DNA(rDNA). The term recombinant is used because DNA from two diff erent sources can be joined together. Hence, genetic engineering is also called as recombinant DNA technology.

Techniques of Genetic Engineering – Basic Requirements

Important discoveries that led to the stepping stone of rDNA technology were

- Presence of plasmid in bacteria that can undergo replication independently along with chromosomal DNA.
- Restriction enzymes cuts or break DNA at specifi c sites and are also called as molecular scissors.
- DNA ligases are the enzymes which help in ligating (joining) the broken DNA fragments.

Gene Cloning

- What reminds to your mind when you hear the word clone? Of course, 'DOLLY' the cloned sheep. The carbon copy of an individual is oft en called a clone. However, more appropriately, a clone means to make a genetically exact copy of an organism.
- In gene cloning, a gene or a piece of DNA fragment is inserted into a bacterial cell where DNA will be multiplied (copied) as the cell divides. A brief outline of the basic steps involved in gene cloning are:
  - Isolation of desired DNA fragment by using restriction enzymes
  - Insertion of the DNA fragment into a suitable vector (Plasmid) to make rDNA Transfer of rDNA into bacterial host cell (Transformation)

- Selection and multiplication of recombinant host cell to get a clone

- Expression of cloned gene in host cell.

Using this strategy several enzymes, hormones and vaccines can be produced

Biotechnology in Medicine

- Using genetic engineering techniques medicinally important valuable proteins or polypeptides that form the potential pharmaceutical products for treatment of various diseases have been developed on a commercial scale.

Pharmaceutical Products developed by rDNA technique

- Insulin used in the treatment of diabetes.

- Human growth hormone used for treating children with growth deficiencies

- Blood clotting factors are developed to treat haemophilia.

- Tissue plasminogen activator is used to dissolve blood clots and prevent heart attack.

- Development of vaccines against various diseases like Hepatitis B and rabies.

Gene Therapy

- Gene therapy refers to the replacement of defective gene by the direct transfer of functional genes into human to treat genetic disease or disorder. The genetic makeup of the patient' cell is altered using recombinant DNA technology. It was first successfully implemented in 1990.

Somatic gene therapy is the replacement of defective gene in Somatic cells.

- **Germ line gene therapy** replacement of defective gene in germ cell (Egg and Sperm)targeted only somatic (non –reproductive) cells. Correction of

genetic defects in Somatic cells may be beneficial to the patient but the Corrected gene may not be carried to the next generation.

Stem Cells

- Our body is composed of over 200 specialised cell types, that can carry out specific functions. eg. neurons or nerve cell that can transmit signals, or heart cells which contract to pump blood or pancreatic cells to secrete insulin. These specialised cells are called as differentiated cell.In contrast to differentiated cells.

- In Contrast to differentiated cells, stem cells are undifferentiated or unspecialised mass of cells. The stem cells are the cells of variable potency. Potency refers to the number of possible fates that a cell can acquire. The two important properties of stem cells that differentiate them from other cells are:

  - Its ability to divide and give rise to more stem cells by self-renewal

  - Its ability to give rise to specialised cells with specific functions by the process of differentiation.

Types of stem cells

- **Embryonic stem cells** can be extracted and cultured from the early embryos. These cells are derived from the inner cell mass of blastocyst. These cells can be developed into any cell in the body.

- **Adult stem cell** or somatic stem cell are found in the neonatal (new born) and adults. They have the ability to divide and give rise to specific cell types. Sources of adult stem cells are amniotic fluid, umbilical cord and bone marrow.

Stem-cell therapy

- Sometimes cells, tissues and organs in the body may be permanently damaged or lost due to genetic condition or disease or injury. In such situations stem cells are used for the treatment of diseases which is called stem-cell therapy. In treating neurodegenerative disorders like Parkinson's disease and Alzheimer's disease neuronal stem cells can be used to replace the damaged or lost neurons.

DNA Finger Printing Technology

- The human genome has 3 billion base pairs. Did you know that the DNA pattern of two individuals cannot be same except for identical twins. Each person's DNA sequence is unique due to the small difference in the base pairs. Therefore, if we want to compare the genetic difference among the two individuals, DNA fingerprinting is the easier and quicker method. This technique was developed by Alec Jeffrey.

- The technique analyses each individual's unique DNA sequences and provides distinctive characteristics of individual which helps in identification. Variable number of tandem repeat sequences (VNTRs) serve as molecular markers for identification.

- In human beings, 99 % of the DNA base sequences are the same and this is called as bulk genomic DNA. The remaining 1 % DNA sequence differs from one individual to another. This 1 % DNA sequence is present as small stretch of repeated sequences which is known as satellite DNA. The number of copies of the repeat sequence also called as VNTRs differs from one individual to another, and results in variation in the size of the DNA segment.

VNTRs illustration of three persons

- As shown in the illustration, the sequence AGCT is repeated six times in fi rst person, fi ve times in second person and seven times in thirdperson. Because of this, DNA segment of third person will be larger in size followed by DNA segment of first person and then the second person. Thus, it is clear that satellite DNA bring about variation within the population. Variation in DNA banding pattern reveals differences among the individuals.

Applications of DNA Fingerprinting

- DNA fingerprinting technique is widely used in forensic applications like crime investigation such as identifying the culprit. It is also used for paternity testing in case of disputes.
- It also helps in the study of genetic diversity of population, evolution and speciation.

Genetically Modified Organisms (GMOs)

- One of the most tremendous development of genetic engineering is the production of genetically modified (GM) plants and animals. Genetic modification refers to the alteration or manipulation of genes in the organisms using rDNA techniques in order to produce the desired characteristics. The DNA fragment inserted is called transgene. Plants or animals expressing a modified endogenous gene or a foregin gene are also known as transgenic organisms.

- The transgenic plants are much stable, with improved nutritional quality, resistant to diseases and tolerant to various environment conditions. Similarly transgenic animalsare used to produce proteins of medicinal importance at low cost and improve livestock quality.

Genetically Modified Plants

Objective	Gene inserted	Achievement
Improved nutritional quality in Rice	Beta carotene gene (In humans, Beta carotene is required for the synthesis of Vitamin A)	Golden Rice (Genetically modified rice can produce beta carotene, that can prevent Vitamin A deficiency)
Increased crop production	Bt gene from bacteria Bacillus thuringiensis. (Bt gene produces a protein that is toxic to insects)	Insect resistant plants (These plants can produce the toxin protein that kills the insects which attack them)

Genetically Modified Animals

Objective	Gene inserted	Achievement
Improved wool quality and production	Genes for synthesis of amino acid, cysteine	Transgenic sheep (gene expressed)
Increased growth in fishes	Salmon or Rainbow trout or Tilapia growth hormone gene	Transgenic fish (gene expressed)

12th zoology

Principles of Inheritance and Variation

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- Genetics is a branch of biology that dealswith the study of heredity and variations. It describes how characteristics and features pass on from the parents to their offsprings in each successive generation. The unit of heredity is known as the gene. Gene is the inherited factor that determines the biological character of an organism. A variation is the degree by which the progeny differs from their parents.

Multiple alleles

- The genetic segregations in Mendelian inheritance reveal that all genes have two alternative forms – dominant and recessive alleles e.g. tall versus dwarf (T and t). The former is the normal allele or wild allele and the latter the mutant allele. A gene can mutate several times producing several alternative forms. When three or more alleles of a gene that control a particular trait occupy the same locus on the homologous chromosome of an organism, they are called multiple alleles and their inheritance is called multiple allelism.

ABO blood types
Multiple allele inheritance of ABO blood groups

- Blood differs chemically from person to person. When two different incompatible blood types are mixed, agglutination (clumping together) of erythrocytes (RBC) occurs. The basis of these chemical differences is due to the presence of antigens (surface antigens) on the membrane of RBC and epithelial cells. Karl Landsteiner discovered two kinds of antigens called antigen 'A' and antigen 'B' on the surface of RBC's of human blood. Based on the presence or absence of these antigens three kinds of blood groups, type 'A', type 'B', and type 'O' (universal donor)were recognized. The fourth and the rarest blood group 'AB' (universal recipient) was discovered in 1902 by two of Landsteiner's students Von De Castelle and Sturli.

- Bernstein in 1925 discovered that the inheritance of different blood groups in human beings is determined by a number of multiple allelic series. The three autosomal alleles located on chromosome 9 are concerned with the determination of blood group in any person. The gene controlling blood

type has been labeled as 'L' (after the name of the discoverer, Landsteiner) or I (from isoagglutination). The I gene exists in three allelic forms, I_A, I_B and I_O. I_A specifies A antigen. I_B allele determines B antigen and I_O allele specifies no antigen. Individuals who possess these antigens in their fluids such as the saliva are called secretors.

- Each allele (I_A and I_B) produces a transferase enzyme. I_A allele produces N-acetyl galactose transferase and can add N-acetyl galactosamine (NAG) and I_B allele encodes for the enzyme galactose transferase that adds galactose to the precursor (i.e. H substances) In the case of I_O/I_O allele no terminal transferase enzyme is produced and therefore called "null" allele and hence cannotadd NAG or galactose to the precursor.

- From the phenotypic combinations it isevident that the alleles IA and IB are dominantto IO, but co-dominant to each other (IA=IB).Their dominance hierarchy can be givenas (IA=IB> IO). A child receives one of threealleles from each parent, giving rise to sixpossible genotypes and four possible bloodtypes (phenotypes). The genotypes are IAIA, IAIO, IBIB, IB IO, IAIB and IO IO.

Genetic basis of the human ABO blood groups

Genotype	ABO bloodgroupphenotype	Antigenspresent onred bloodcell	Antibodiespresent in blood plasma
I_AI_A	Type A	A	Anti -B
I_AI_o	Type A	A	Anti -B
I_BI_B	Type B	B	Anti -A
I_BI_o	Type B	B	Anti -A
I_AI_B	Type AB	A and B	Neither Anti -A nor Anti-B
I_oI_o	Type O	Neither A nor B	Anti –A and anti -B

Genetic control of Rh factor
Fisher and Race hypothesis:

- Rh factor involves three different pairs of alleles located on three different closely linked loci on the chromosome pair. This system is more commonly in use today, and uses the 'Cde' nomenclature.

Wiener Hypothesis

- Wiener proposed the existence of eight alleles (R1, R2, R0, Rz, r, r1, r11, ry) at a single Rh locus. All genotypes carrying a dominant 'R allele' (R1, R2 ,R0 ,Rz) will produce Rh+positivephenotype and double recessive genotypes (rr, rr1, rr11, rry) will give rise to Rh-negative phenotype.

Incompatibility of Rh - Factor – Erythroblastosis foetalis

- Rh incompatability has great significance in child birth. If a woman is Rh negative andthe man is Rh positive, the foetus may be Rh positive having inherited the factor fromits father. The Rh negative mother becomes sensitized by carrying Rh positive foetus within her body. Due to damage of blood vessels, during child birth, the mother's immune system recognizes the Rh antigens and gets sensitized. The sensitized mother produces Rh antibodies. The antibodies are IgG type which are small and can cross placenta and enter the foetal circulation. By the time the mother gets sensitized and produce anti 'D' antibodies, the child is delivered.

- Usually no effects are associated with exposure of the mother to Rh positive antigen during the first child birth, subsequent Rh positive children carried by the same mother, may be exposed to antibodies produced by the mother against Rh antigen, which are carried across the placenta into the foetal blood circulation. This causes haemolysis offoetal RBCs resulting in haemolytic jaundice and anaemia. This condition is known as Erythoblastosisfoetalis or Haemolyticdisease of the new born (HDN).

Inheritance of Y- linked genes

- Genes in the non-homologous region of the Y-chromosome are inherited directly from male to male. In humans, the Y-linked or holandric genes for hypertrichosis (excessive development of hairs on pinna of the ear) are transmitted directly from father to son, because males inherit the Y chromosome from the father. Female inherits only X chromosome from the father and are not affected.

Genetic Disorders:

- A genetic disorder is a disease or syndrome that is caused by an abnormalityin an individual DNA. Abnormalities can range from a small mutation in a single gene to the addition or subtraction of an entire chromosome or even a set ofchromosomes. Genetic disorders are of two types namely, Mendelian disorders and chromosomal disorders.

Mendelian disorders

- Alteration or mutation in a single gene causes Mendelian disorders. These disorders are transmitted to the offsprings on the same line as the Mendelian pattern of inheritance. Some examples for Mendelian disorders are Thalassemia, albinism, phenylketonuria, sickle cell anaemia, Huntington's chorea, etc., These disorders may be dominant or recessive and autosomal or sex linked.

Thalassemia

- Thalassemia is an autosomal recessive disorder. It is caused by gene mutation resultingin excessive destruction of RBC's due to the formation of abnormal haemoglobin molecules.

- Normally haemoglobin is composed of four polypeptide chains, two alpha and two betaglobin chains. Thalassemia patients have defects in either the alpha or beta globin chain causing the production of abnormal haemoglobin molecules resulting in anaemia.

- Thalassemia is classified into alpha and beta based on which chain of haemoglobin molecule is affected. It is controlled by two closely linked genes HBA1 and HBA2 on chromosome 16. Mutation or deletion of one or more of the four alpha gene alleles causes Alpha Thalassemia. In Beta Thalassemia, production of beta globin chain is affected. It is controlled by a single gene (HBB) on chromosome 11. It is the most common type of Thalassemia and is also known as Cooley's anaemia. In this disorder the alphachain production is increased and damages the membranes of RBC.

UNIT – 5 - Molecular Genetics

- Mendel's theory dispelled the mystery of why traits seemed to appear anddisappear magically from one generation to the next. Mendel's work reveals the patterns of heredity and reflect the transmission of evolved information from parents to offspring. This information is located on the chromosomes. One of the most advanced realizations of human knowledge was that our unique characteristics are encoded within molecules of DNA. The discovery that DNA is the genetic material left several questions unanswered.

- How is the information in DNA used? Scientists now know that DNA directs the construction of proteins. Proteins determine the shapes of cells and the rate of chemical reactions, such as those that occur during metabolism and photosynthesis. The hereditary nature of every living organism is defined by its genome, which consists of a long sequence of nucleic acids that provide the information needed to construct the organism. The genome contains the complete set of hereditary information for any organism. The genome may be divided into a number of different nucleic acid molecules. Each of the nucleic acid molecule may contain large number of genes. Each gene is a sequence within the nucleic acid that represents a single protein. In this chapter we will discuss the structure of DNA, its replication, the process of making RNA from DNA (transcription), the genetic code that determines the sequence of amino acid in protein synthesis (translation), regulation of gene expression and the essentials of human genome sequencing.

Gene as the functional unit of inheritance

- A gene is a basic physical and functional unit of heredity. The concept of the gene was first explained by Gregor Mendel in 1860's. He never used the term 'gene'. Hecalled it 'factor'. In 1909, the Danish biologist Wilhelm Johannsen, coined the term 'gene', that was referred to discrete determiners of inherited characteristics.

- According to the classical concept of gene introduced by Sutton in 1902, genes have been defined as discrete particles that follow Mendelian rules of inheritance, occupy a definite locus in the chromosome and are responsible

for the expression of specific phenotypic character. They show the following properties:

- Number of genes in each organism is more than the number of chromosomes; hence several genes are located on the same chromosome.
- The genes are arranged in a single linear order like beads on a string.
- Each gene occupies a specific position called locus.
- Genes may exist in several alternate forms called alleles.
- Genes may undergo sudden change in positions and composition called mutations.
- Genes are capable of self-duplication producing their own copies.

One gene-one enzyme hypothesis:
The experiments of George Beadle and Edward Tatum in the early 1940's on Neurospora crassa (the red bread mould) led them to propose one geneone enzyme hypothesis, which states that one gene controls the production of one enzyme.

One gene-one polypeptide hypothesis:
It was observed that an enzyme may be composed of more than one polypeptide chain and a gene can code for only one polypeptide chain. Thus one gene-one polypeptide hypothesis states that one gene controls the production of only one polypeptide chain of an enzyme molecule.

In search of the genetic material

- As early as 1848, Wilhelm Hofmeister, a German botanist, had observed that cell nuclei organize themselves into small, rod like bodies during mitosis called chromosomes. In 1869, Friedrich Miescher, a Swiss physician, isolated a substance from the cell nuclei and called it as nuclein. It was renamed as nucleic acid by Altman (1889), and is now known as DNA. By 1920, it became clear that chromosomes are made up of proteins and DNA. Many experimentswere carried out to study the actual carriers of genetic information. Griffith's experiment proved that DNA is the genetic material

which has been dealt in class XI. Bacterial transformation experiments provided the first proof that DNA is the genetic material.

- However, he could not understand the cause of bacterial transformation, and the biochemical nature of genetic material was not defined from his experiments.

- Later, Oswald Avery, Colin Macleod and Maclyn McCarty in 1944 repeated Griffith's experiments in an 'in vitro' system in order to identify the nature of the transforming substance responsible for converting a nonvirulent strain into virulent strain. They observed that the DNA, RNA and proteins isolated from the heat-killed S-strain when added to R-strain changed their surface character from rough to smooth and also made them pathogenic But when the extract was treated with DNase (an enzyme which destroys DNA) the transforming ability was lost. RNase (an enzyme which destroys RNA) and proteases(an enzyme which destroys protein) did not affect the transformation. Digestion with DNase inhibited transformation suggesting that the DNA caused the transformation. These experiments suggested that DNA and not proteins is the genetic material. The phenomenon, by which DNA isolated from one type of cell (R – strain), when introduced into another type (S-strain), is able to retain some of the properties of the R - strain is referred to as transformation.

DNA is the genetic material

- Many biologists despite the earlier experiments of Griffith, Avery and others, still believed that protein, not DNA, was the hereditary material in a cell. As eukaryotic chromosomes consist of roughly equal amounts of protein and DNA, it was said that only a protein had sufficient chemical diversity and complexity to encode the information required for genetic material. In 1952, however, the results of the Hershey-Chase experiment finally provided convincing evidence that DNA is the genetic material.

Properties of genetic material (DNA versus RNA)

- The experiment by Hershey and Chase clearly indicates that it is DNA that acts as a genetic material. However, in some viruses like Tobacco mosaic virus (TMV), bacteriophage θB, RNA acts as the genetic material. A molecule that can act as a genetic material should have the following properties:

Self Replication:

- It should be able to replicate. According to the rule of base pairing and complementarity, both nucleic acids (DNA and RNA) have the ability to direct duplications. Proteins fail to fulfil this criteria.

Stability:

- It should be stable structurally and chemically. The genetic material should be stable enough not to change with different stages of life cycle, age or with change in physiology of the organism. Stability as one of property of genetic material was clearly evident in Griffith's transforming principle. Heat which killed the bacteria did not destroy some of the properties of genetic material. In DNA the two strands being complementary, if separated (denatured) by heating can come together (renaturation) when appropriate condition is provided. Further 2' OH group present at every nucleotide in RNA is a reactive group that makes RNA liable and easily degradable. RNA is also known to be catalytic and reactive. Hence, DNA is chemically more stable and chemically less reactive when compared to RNA. Presence of thymine instead of uracil in DNA confers additional stability to DNA.

Information storage:

- It should be able to express itself in the form of 'Mendelian characters'. RNA can directly code for protein synthesis and can easily express the characters. DNA, howeverdepends on RNA for synthesis of proteins. Both DNA and RNA can act as a genetic material, but DNA being more stable stores the genetic information and RNA transfers the genetic information.

Variation through mutation:

- It should be able to mutate. Both DNA and RNA are able to mutate.RNA being unstable, mutates at a faster rate. Thus viruses having RNA genome with shorter life span can mutate and evolve faster.

- The above discussion indicates that both RNA and DNA can function as a genetic material. DNA is more stable, and is preferred for storage of genetic information.

- Chromosomes are carriers of genes which are responsible for various characters from generation to generation. Du Praw (1965) proposed a single stranded model (unineme), as a long coiled molecule which is associated with histone proteins in eukaryotes. Plants and animals have more DNA than bacteria and must fold this DNA to fit into the cell nucleus. In prokaryotes such as *E. coli* though they do not have defined nucleus, the DNA is not scattered throughout the cell. DNA (being negatively charged) is held with some proteins (that have positive charges) in a region called the nucleoid. The DNA as a nucleoid is organized into large loops held by protein. DNA of prokaryotes is almost circular and lacks chromatin organization, hence termed genophore.

Transcription

- The process of copying genetic information from one strand of DNA into RNA is termed transcription. This process takes place in presence of DNA dependent RNA polymerase.

- In some retroviruses that contain RNA as the genetic material (e.g, HIV), the flow of information is reversed. RNA synthesizes DNA by reverse transcription, then transcribed into mRNA by transcription and then into proteins by translation.

- For a cell to operate, its genes must be expressed. This means that the gene products, whether proteins or RNA molecules must be made. The RNA that carries genetic information encoding a protein from genes into the cell is known as messenger RNA (mRNA). For a gene to be transcribed, the DNA which is a double helix must be pulled apart temporarily, and RNA is synthesized by RNA polymerase. This enzyme binds to DNA at the start of a gene and opens the double helix. Finally, RNA molecule is synthesized. The nucleotide sequence in the RNA is complementary to the DNA template strand from which it is synthesized.

- Both the strands of DNA are not copied during transcription for two reasons. If both the strands act as a template, they would code for RNA with different sequences. This in turn would code for proteins with different amino acid sequences. This would result in one segment of DNA coding for two different proteins, hence complicate the genetic information transfer machinery. If two RNA molecules were produced

simultaneously, double stranded RNA complementary to each other would be formed. This would prevent RNA from being translated into proteins.

Transcription unit and gene

- A transcriptional unit in DNA is defined by three regions, a promoter, the structural gene and a terminator. The promoter is located towards the 5' end. It is a DNA sequence that provides binding site for RNA polymerase. The presence of promoter in a transcription unit, defines the template and coding strands. The terminator region located towards the 3' end of the coding strand contains a DNA sequence that causes the RNA polymerase to stop transcribing. In eukaryotes the promoter has AT rich regions called TATA box (Goldberg-Hogness box) and in prokaryotes this region is called Pribnow box. Besides promoter,eukaryotes also require an enhancer.

- The two strands of the DNA in the structural gene of a transcription unit have oppositepolarity. DNA dependent RNA polymerase catalyses the polymerization in only one direction, the strand that has the polarity 3' 5' acts as a template, and is called the template strand. The other strand which has the polarity 5' 3' has a sequence same as RNA (except thymine instead of uracil) and is displaced during transcription. This strand is called coding strand.

- The structural gene may be monocistronic (eukaryotes) or polycistronic (prokaryotes). In eukaryotes, each mRNA carries only a single gene and encodes information for only a singleprotein and is called monocistronic mRNA. In prokaryotes, clusters of related genes, known asoperon, often found next to each other on the chromosome are transcribed together to give a single mRNA and hence are polycistronic. Before starting transcription, RNA polymerase binds to the promoter, a recognition sequence in front of the gene. Bacterial (prokaryotic) RNA polymerase consists of two major components, the core enzyme and the sigma subunit. The core enzyme (β1, β, and α) is responsible for RNA synthesis whereas a sigma subunit isresponsible for recognition of the promoter. Promoter sequences vary in different organisms.

- RNA polymerase opens up the DNA to form the transcription bubble. The core enzyme moves ahead, manufacturing RNA leaving the sigma subunit behind at the promoter region. The end of a gene is marked by a terminator

sequence that forms a hair pin structure in the RNA. The sub-class of terminators require a recognition protein, known as rho (ρ), to function.

Genetic Code

- DNA is the genetic material that carries genetic information in a cell and from generation to generation. At this stage, an attempt will be made to determine in what manner the genetic information exists in DNA molecule? Are they written in coded language on a DNA molecule? If they occur in the language of codes what is the nature of genetic code? The translation of proteins follows the triplet rule; a sequence of three mRNA base (a codon) designates one of the 20 different kinds of amino acids used in protein synthesis.

- Genetic code is the sequence relationship between nucleotide in genes (or mRNA) and the amino acids in the proteins they encode. There are 64 possible triplets, and 61 of them are used to represent amino acids. The remaining three triplet codons are termination signals for polypeptide chains. Since there are only 20 amino acids involved in protein synthesis, most of them are encoded by more than one triplet. Two things make this multiple (degenerate) coding possible. First, there is more than one tRNA for most amino acids. Each tRNA has a different anticodon. Second, this pairing is highly specific for the first two portions on the codon, permitting Watson and Crick base pairs (A – U and G - C) to be formed. But at the third position there is a great deal of flexibility as to which base pairs are acceptable. Most part of the genetic code is universal, being the same in prokaryotes and eukaryotes.

- The order of base pairs along DNA molecule controls the kind and order of amino acids found in the proteins of an organism. This specific order of base pairs is called genetic code, the blue print establishing the kinds of proteins to be synthesized which makes an organism unique.

- Marshall Nirenberg, Severo Ochoa (enzyme polynucleotide phosphorylase called Ochoa'senzyme), Hargobind Khorana, Francis Crick and many others have contributed significantlyto decipher the genetic code. The order in which bases are arranged in mRNA decides the order in which amino acids are arranged in proteins.

The salient features of genetic code are as follows:

- The genetic codon is a triplet code and 61 codons code for amino acids and 3 codons do not code for any amino acid and function as stop codon (Termination).

- The genetic code is universal. It means that all known living systems use nucleic acids and the same three base codons (triplet codon) direct the synthesis of protein from amino acids. For example, the mRNA (UUU) codon codes for phenylalanine in all cells of all organisms. Some exceptions are reported in prokaryotic, mitochondrial and chloroplast genomes. However similarities are more common than differences.

- A non-overlapping codon means that the same letter is not used for two different codons. For instance, the nucleotide sequence GUU GUC represents only two codons.

- It is comma less, which means that the message would be read directly from one end to the other i.e., no punctuation are needed between two codes.

- A degenerate code means that more than one triplet codon could code for a specific amino acid. For example, codons GUU, GUC, GUA and GUG code for valine.

- Non-ambiguous code means that one codon will code for one amino acid.

- The code is always read in a fixed direction i.e. from 5'→3' direction called polarity.

- AUG has dual functions. It acts as a initiator codon and also codes for the amino acid methionine.

- UAA, UAG (tyrosine) and UGA (tryptophan) codons are designated as termination (stop) codons and also are known as "non-sense" codons.

Mutation and genetic code

- Comparative studies of mutations (sudden change in a gene) and corresponding alteration in amino acid sequence of specific protein have confirmed the validity of the genetic code. The relationship between genes

and DNA are best understood by mutation studies. The simplest type of mutation at the molecular level is a change in nucleotide that substitutes one base for another. Such changes are known as base substitutions which may occur spontaneously or due to the action of mutagens. A well studied example is sickle cell anaemia in humans which results from a point mutation of an allele of β-haemoglobin gene (βHb). A haemoglobin molecule consists of four polypeptide chains of two types, two α chains and two β-chains. Each chain has a heme group on its surface. The heme groups are involved in the binding of oxygen. The human blood disease, sickle cell anaemia is due to abnormal haemoglobin. This abnormality in haemoglobin is due to a single base substitution at the sixth codon of the beta globin gene from GAG to GTG in β -chain of haemoglobin. It results in a change of amino acid glutamic acid to valine at the 6th position of the β -chain. This is the classical example of point mutation that results in thechange of amino acid residue glutamic acid to valine. The mutant haemoglobinundergoes polymerisation under oxygen tension causing the change in the shape of the RBC from biconcave to a sickle shaped structure.

Regulation of gene expression

- We have previously established how DNA is organized into genes, how genes store genetic information, and how this information is expressed. We now consider the most fundamental issues in molecular genetics. How is genetic expression regulated? Evidence in support of the idea that genes can be turned on and off is very convincing. Regulation of gene expression has been extensively studied in prokaryotes, especially in E. coli. Gene expression can be controlled or regulated at transcriptional or post transcriptional or translational level. Here, we are going to discuss regulation of gene expression at transcriptional level. Usually, small extracellular or intracellular metabolites trigger initiation or inhibition of gene expression. The clusters of gene with related functions are called operons. They usually transcribe single mRNA molecules. In E.coli, nearly 260 genes are grouped into 75 different operons.

Structure of the operon:

- Each operon is a unit of gene expression and regulation and consistsof one or more structural genes and an adjacent operator gene that controls transcriptional activity of the structural gene.

- The structural gene codes for proteins, rRNA and tRNA required by the cell.

- Promoters are the signal sequences in DNA that initiate RNA synthesis. RNA polymerase binds to the promoter prior to the initiation of transcription.

- The operators are present between the promoters and structural genes. Therepressor protein binds to the operator region of the operon.

The Lac (Lactose) operon:

- The metabolism of lactose in E.coli requires three enzymes – permease, β-galactosidase (β-gal) and transacetylase. The enzyme permease is needed for entry of lactose into the cell, β-galactosidase brings about hydrolysis of lactose to glucose and galactose, while transacetylase transfers acetyl group from acetyl Co A to β-galactosidase.

- The lac operon consists of one regulator gene ('i' gene refers to inhibitor) promoter sites (p), and operator site (o). Besides these, it has three structural genes namely lac z,y and lac a. The lac 'z' gene codes for β-galactosidase, lac 'y' gene codes for permease and 'a' gene codes for transacetylase. Jacob and Monod proposed the classical model of Lac operon to explain gene expression andregulation in E.coli. In lac operon, a polycistronic structural gene is regulated by a common promoter and regulatory gene. When the cell is using its normal energy source as glucose, the 'i' gene transcribes a repressor mRNA and after its translation, a repressor protein is produced.

- It binds to the operator region of the operon and prevents translation, as a result, β-galactosidase is not produced. In the absence of preferred carbon source such as glucose, if lactose is available as an energy source for the bacteria then lactose enters the cell as a result of permease enzyme. Lactose acts as an inducer and interacts with the repressor to inactivate it.

- The repressor protein binds to the operator of the operon and prevents RNA polymerasefrom transcribing the operon. In the presence of inducer, such as lactose or allolactose, the repressor is inactivated by interaction with the inducer. This allows RNA polymerase to bind to the promotor site and transcribe the operon to produce lac mRNA which enables formation of all the required enzymes needed for lactose metabolism. This regulation of lac operon by the repressor is an example of negative control of transcription initiation. Lacoperon is also under the control of positive regulation as well.

Human GenomeProject (HGP)

- The international human genome project was launched in the year 1990. It was a mega project and took 13 years to complete. The human genome is about 25 times larger than the genome of any organism sequenced to date and is the first vertebrate genome to be completed. Human genome is said to have approximately 3×109 bp. HGP was closely associated with the rapid development of a new area in biology called bioinformatics.

Goals and methodologies of Human Genome Project

The main goals of Human Genome Project are as follows

- Identify all the genes (approximately 30000) in human DNA.
- Determine the sequence of the three billion chemical base pairs that makeup the human DNA.
- To store this information in databases.
- Improve tools for data analysis.
- Transfer related technologies to other sectors, such as industries.
- Address the ethical, legal and social issues (ELSI) that may arise from the project.

- The methodologies of the Human Genome Project involved two major approaches. One approach was focused on identifying all the genes that are expressed as RNA (ETSS – Expressed Sequence Tags). The other approach was sequence annotation. Here, sequencing the whole set of genome was taken, that contains all the coding and non-coding sequences and later assigning different regions in the sequences with functions. For sequencing, the total DNA froma cell is isolated and converted into random fragments of relatively smaller sizes and cloned in suitable hosts using specialized vectors. This cloning results in amplification of pieces of DNA fragments so that it could subsequently be sequenced with ease. Bacteria and yeast are two commonly used hosts and these vectors are called as BAC (Bacterial Artificial Chromosomes) and YAC (Yeast Artificial Chromosomes). The fragments are sequenced using automated DNA sequencers (developed by Frederick Sanger). The sequences are then arranged based on few overlapping regions, using specialized computer based programs. These sequences were subsequently annotated and are assigned to each chromosome. The genetic and physical maps on the genome are assigned using information on polymorphism of restriction endonuclease recognition

sites and some repetitive DNA sequences, called microsatellites. The latest method of sequencing even longer fragments is by a method called Shotgun sequencing using super computers, which has replaced the traditional sequencing methods.

Salient features of Human Genome Project:

- Although human genome contains 3 billion nucleotide bases, the DNA sequences that encode proteins make up only about 5% of the genome.
- An average gene consists of 3000 bases, the largest known human gene being dystrophin with 2.4 million bases.
- The function of 50% of the genome is derived from transposable elements such as LINE and ALU sequence.
- Genes are distributed over 24 chromosomes. Chromosome 19 has the highest gene density. Chromosome 13 and Y chromosome have lowest gene densities
- The chromosomal organization of humangenes shows diversity.
- There may be 35000-40000 genes in thegenome and almost 99.9 nucleotide basesare exactly the same in all people.
- Functions for over 50 percent of thediscovered genes are unknown.
- Less than 2 percent of the genome codesfor proteins.
- Repeated sequences make up very largeportion of the human genome. Repetitivesequences have no direct coding functionsbut they shed light on chromosomestructure, dynamics and evolution(genetic diversity).
- Chromosome 1 has 2968 genes whereaschromosome 'Y' has 231 genes.
- Scientists have identified about 1.4million locations where single baseDNA differences (SNPs – Singlenucleotidepolymorphism – pronounce as'snips') occur in humans. Identification of'SNIPS' is helpful in finding chromosomallocations for disease associated sequencesand tracing human history

Applications and future challenges

- The mapping of human chromosomes is possible to examine a person's DNA and to identify genetic abnormalities. This is extremely useful in diagnosing diseases and to provide genetic counselling to those planning to have children. This kind of information would also create possibilities for new gene therapies. Besides providing clues to understand human biology, learning about non-human organisms, DNA sequences can lead to an understanding of their natural capabilities that can be applied towards solving challenges in healthcare, agriculture, energy production and environmental remediation. A new era of molecular medicine,characterized by looking into the most fundamental causes of disease than treating the symptoms will be an important advantage.

    - Once genetic sequence becomes easierto determine, some people may attemptto use this information for profit or forpolitical power.

    - Insurance companies may refuse to insurepeople at 'genetic risk' and this would savethe companies the expense of future medical bills incurred by 'less than perfect' people.

    - Another fear is that attempts are beingmade to "breed out" certain genes ofpeople from the human population inorder to create a 'perfect race'.

Pharmacogenomics is the study of how genes affect a person's response to drugs. This relatively new field combines pharmacology (the science of drugs) and genomics (the study of genes and their functions) to develop effective, safe medications and doses that will be tailored to a person's genetic makeup.

DNA fingerprinting technique

- The DNA fingerprinting technique was first developed by Alec Jeffreys in 1985 (Recipient of the Royal Society's Copley Medal in 2014). Each of us have the same chemical structure of DNA. But there are millions of differences in the DNA sequence of base pairs. This makes the uniqueness among us so that each of us except identical twins is different from each other genetically. The DNA of a person and finger prints are unique. There are 23 pairs of human chromosomes with 1.5 million pairs of genes. It is a well known fact that genes are segments of DNA which differ in the sequence of their nucleotides. Not all segments of DNA code for proteins,

some DNA segments have a regulatory function, while others are intervening sequences (introns) and still others are repeated DNA sequences. In DNA fingerprinting, short repetitive nucleotide sequences are specific for a person. These nucleotide sequences are called as variable number tandem repeats (VNTR).The VNTRs of two persons generally show variations and are useful as genetic markers.

- DNA finger printing involves identifying differences in some specific regions in DNA sequence called repetitive DNA, because in these sequences, a small stretch of DNA is repeated many times. These repetitive DNA are separated from bulk genomic DNA as different peaks during density gradient centrifugation. The bulk DNA forms a major peak and the other small peaks are referred to as satellite DNA. Depending on base composition (A : T rich or G : C rich), length of segment and number of repetitive units, the satellite DNA is classified into many sub categories such as micro-satellites, mini-satellites, etc., These sequences do not code for any proteins, but they form a large portion of human genome. These sequences show high degree of polymorphism and form the basis of DNA fingerprinting (Fig. 5.15). DNA isolated from blood, hair, skin cells, or other genetic evidences left at the scene of a crime can be compared through VNTR patterns, with the DNA of a criminal suspect to determine guilt or innocence. VNTR patterns are also useful in establishing the identity of a homicide victim, either from DNA found as evidence or from the body itself.

The Steps in DNA Fingerprinting technique

Extraction of DNA

- The process of DNA fingerprinting starts with obtaining a sample of DNA from blood, semen, vaginal fluids, hair roots, teeth, bones, etc.,

Polymerase chain reaction (PCR)

- In many situations, there is only a small amount of DNA available for DNA fingerprinting. If needed many copies of the DNA can be produced by PCR (DNA amplification).

Fragmenting DNA

- DNA is treated with restriction enzymes which cut the DNA into smaller fragments at specific sites.

Separation of DNA by electrophoresis

- During electrophoresis in an agarose gel, the DNA fragments are separated into bands of different sizes. The bands of separated DNA are sieved out of the gel using a nylon membrane (treated with chemicals that allow for it to break the hydrogen bonds of DNA so there are single strands).

Denaturing DNA

The DNA on gels is denatured by using alkaline chemicals or by heating.

Blotting

- The DNA band pattern in the gel istransferred to a thin nylon membraneplaced over the 'size fractionated DNAstrand' by Southern blotting.

Using probes to identify specific DNA

- A radioactive probe (DNA labeled witha radioactive substance) is added to theDNA bands. The probe attaches by basepairing to those restriction fragments thatare complementary to its sequence. Theprobes can also be prepared by using either'fluorescent substance' or 'radioactiveisotopes'.

Hybridization with probe

- After the probe hybridizes and the excessprobe washed off, a photographic filmis placed on the membrane containing'DNA hybrids'.

Exposure on film to make a genetic/DNA Fingerprint

- The radioactive label exposes the filmto form an image (image of bands)corresponding to specific DNA bands. The thick and thin dark bands form a pattern of bars which constitutes a genetic fingerprint.

Application of DNAfinger printing

- **Forensic analysis** - It can be used in theidentification of a person involved incriminal activities, for settling paternityor maternity disputes, and in determiningrelationships for immigration purposes.

- **Pedigree analysis** – inheritance patternof genes through generations and fordetecting inherited diseases.

- **Conservation of wild life** – protection ofendangered species. By maintaining DNArecords for identification of tissues of thedead endangered organisms.

- **Anthropological studies**–It is useful indetermining the origin and migration of human populations and genetic diversities.

UNIT – 6- Evolution:

Origin of life – Evolution of life forms

- Theory of special creation states that life was created by a supernatural power, respectfully referred to as "God". According to Hinduism, Lord Brahma created the Earth. Christianity, Islam and most religions believe that God created the universe, the plants and the animals.

- According to the theory of spontaneous generation or Abiogenesis, living organisms originated from non-living materials and occurred through stepwise chemical and molecular evolution over millions of years. Thomas Huxley coined the term abiogenesis.

- Big bang theory explains the origin of universe as a singular huge explosion in physical terms. The primitive earth had no proper atmosphere, but consisted of ammonia, methane, hydrogen and water vapour. The climate of the earth was extremely high. UV rays from the sun split up water molecules into hydrogen and oxygen. Gradually the temperature cooled and the water vapour condensed to form rain. Rain water filled all the depressions to form water bodies. Ammonia and methane in the atmosphere combined with oxygen to form carbon-dioxide and other gases.

Coacervates (large colloidal particles that precipitate out in aqueous medium) are the first pre-cells which gradually transformed into living cells.

- According to the theory of biogenesis life arose from pre-existing life. The term biogenesis also refers to the biochemical process of production of living organisms This term was coined by Henry Bastian.

- According to the theory of chemical evolution primitive organisms in the primordial environment of the earth evolved spontaneously from inorganic substances andphysical forces such, as lightning, UV radiations, volcanic activities, etc.,., Oparin (1924) suggested that the organic compounds could have undergone a series of reactions leading to more complex molecules. He proposed that the molecules formed colloidal aggregates or 'coacervates' in an aqueous environment. The coacervates were able to absorb and assimilate organic compounds from the environment. Haldane (1929) proposed that the primordial sea served as a vast chemical laboratory powered by solar energy. The atmosphere was oxygen free and

the combination of CO2, NH3 and UV radiations gave rise to organic compounds. The sea became a 'hot' dilute soup containing large populations of organic monomers and polymers. They envisaged that groups of monomers and polymers acquired lipid membranes and further developed into the first living cell. Haldane coined the term prebiotic soup and this became the powerful symbol of the Oparin-Haldane view on the origin of life (1924-1929).

- Oparin and Haldane independently suggested that if the primitive atmosphere was reducing and if there was appropriate supply of energy such as lightning or UV light then a wide range of organic compounds can be synthesized.

Evidences for biological evolution
Paleontological evidences

- Paleontology is the study of prehistoric life through fossils. Fossils are described as the true witnesses of evolution or documents of various geological strata of evolution. Fossilization is the process by which plant and animal remains are preserved in sedimentary rocks. They fall under three main categories.

Actual remains :

- The original hard parts such as bones, teeth or shells are preserved as such in the earth's atmosphere. This is the most common method of fossilization. When marine animals die, their hard parts such as bones, shells, etc., are covered with sediments and are protected from further deterioration. They get preserved as such as they are preserved in vast ocean; the salinity in them prevents decay. The sediments become hardened to form definite layers or strata. For example, Woolly Mammoth that lived 22 thousand years ago were preserved in the frozen coast of Siberia as such. Several human beings and animals living in the ancient city of Pompeii were preserved intact by volcanic ash which gushed out from Mount Vesuvius.

Petrifaction

- When animals die the original portion of their body may be replaced molecule for molecule by minerals and the original substance being lost through disintegration. This method of fossilization is called petrifaction. The principle minerals involved in this type fossilization are iron pyrites, silica, calcium carbonate and bicarbonates of calcium and magnesium.

Natural moulds and casts

- Even after disintegration, the body of an animal might leave indelible impression on the soft mud which later becomes hardened into stones. Such impressions are called moulds. The cavities of the moulds may get filled up by hard minerals and get fossilized, which are called casts. Hardened faecal matter termed as coprolites occur as tiny pellets. Analysis of the coprolites enables us to understand the nature of diet the pre-historic animals thrived on.

Embryological evidences

- Embryology deals with the study of the development of individual from the egg to the adult stage. A detailed study of the embryonic development of different forms makes us to think that there is a close resemblance during development.

- The development of heart in all vertebrates follows the same pattern of development as a pair of tubular structures that later develop into two chambered heart in fishes, three chambered in amphibians and in most reptiles and four chambered in crocodiles, birds and mammals; indicating a common ancestry for all the vertebrates,

- Hence scientists in the 19th century concluded that higher animals during their embryonic development pass through stages of lower animals (ancestors). Ernst Von Haeckel, propounded the "biogenetic law ortheory of recapitulation" which states that the life history of an individual (ontogeny) briefly repeats or recapitulates the evolutionary history of the race (phylogeny). In other words "Ontogeny recapitulates Phylogeny". The embryonic stages of a higher animal resemble the adult stage of its ancestors. Appearance of pharyngeal gill slits, yolk sac and the appearance of tail in human embryos are some of the examples. The biogenetic law is not universal and it is now thought that animals do not recapitulate the adult stage of any ancestors. The human embryo recapitulates the embryonic history and not the adult history of the organisms.

- The comparative study of the embryo of different animals shows structural similarities among themselves. The embryos of fish, salamander, tortoise, chick and human start life as a single cell, the zygote, and undergo cleavage to produce the blastula, change to gastrula and are triploblastic. This

indicates that all the above said animals have evolved from a common ancestor.

Molecular evidences

- Molecular evolution is the process of change in the sequence composition of molecules such as DNA, RNA and proteins across generations. It uses principles of evolutionary biology and population genetics to explain patterns in the changes of molecules.

- One of the most useful advancement in the development of molecular biology is proteins and other molecules that control life processes are conserved among species. A slight change that occurs over time in these conserved molecules (DNA, RNA and protein) are often called molecular clocks. Molecules that have been used to study evolution are cytochrome c (respiratory pathway) and rRNA (protein synthesis).

Theories of biological evolution
Lamarck's theory

- Jean Baptiste de Lamarck, was the first to postulate the theory of evolution in his famous book 'Philosophie Zoologique' in the year 1809. The two principles of Lamarckian theory are:

- **The theory of use and disuse–** Organsthat are used often will increase in size and those that are not used will degenerate. Neck in giraffe is an example of use and absence of limbs in snakes is an example for disuse theory.

- **The theory of inheritance of acquired characters** - Characters that are developed during the life time of an organism are called acquired characters and these are then inherited.

The main objection to Lamarckism

- Lamarck's "Theory of Acquired characters" was disproved by August Weismann who conducted experiments on mice for twenty generations by cutting their tails and breeding them. All mice born were with tail. Weismann proved that change in the somatoplasm will not be transferred to the next generation but changes in the germplasm will be inherited.

Neo-Lamarckism

- The followers of Lamarck (Neo-Lamarckists) like Cope, Osborn, Packard and Spencer tried to explain Lamarck's theory on a more scientific basis. They considered that adaptations are universal. Organisms acquire new structures due to their adaptations to the changed environmental conditions. They argued that external conditions stimulate the somatic cells to produce certain 'secretions' which reach the sex cells through the blood and bring about variations in the offspring.

Darwin's theory of Natural Selection

- Charles Darwin explained the theory of evolution in his book 'The Origin of Species by Natural Selection'. During his journey around the Earth, he made extensive observations of plants and animals. He noted a huge variety and remarkable similarities among organisms and their adaptive features to cope up to their environment. He proved that fittest organisms can survive and leave more progenies than the unfit ones through natural selection.

Darwin's theory was based on several facts, observations and influences. They are:

Over production (or) prodigality of production

- All living organisms increase their population in larger number. For example, Salmon fish produces about 28 million eggs during breeding season and if all of them hatch, the seas would be filled with salmon in few generations. Elephant, the slowest breeder that can produce six young ones in its life time can produce 6 million descendants at the end of 750 years in the absence of any check.

Struggle for existence

- Organisms struggle for food, space and mate. As these become a limiting factor, competition exists among the members of the population. Darwin denoted struggle for existence in three ways –

- Intra specific struggle between the same species for food, space and mate.

- Inter specific struggle with different species for food and space.

- Struggle with the environment to cope with the climatic variations, flood, earthquakes, drought, etc.,

Universal occurrence of variations

- No two individuals are alike. There are variations even in identical twins. Even the children born of the same parents differ in colour, height, behavior, etc., The useful variationsfound in an organism help them to overcome struggle and such variations are passed on to the next generation.

Origin of species by Natural Selection

- According to Darwin, nature is the most powerful selective force. He compared origin of species by natural selection to a small isolated group. Darwin believed that the struggle for existence resulted in the survival of the fittest. Such organisms become better adapted to the changed environment.

Objections to Darwinism

Some objections raised against Darwinism were –

- Darwin failed to explain the mechanism of variation.
- Darwinism explains the survival of the fittest but not the arrival of the fittest.
- He focused on small fluctuating variations that are mostly non-heritable.
- He did not distinguish between somatic and germinal variations.
- He could not explain the occurrence of vestigial organs, over specialization of some organs like large tusks in extinct mammoths, oversized antlers in the extinct Irish deer, etc.,

Neo Darwinism

- Neo Darwinism is the interpretation of Darwinian evolution through Natural Selection as it has been modified since it was proposed. New facts and discoveries about evolution have led to modifications of Darwinism and is supported by Wallace, Heinrich, Haeckel, Weismann and Mendel. This theory emphasizes the change in the frequency of genes in population arises due to mutation, variation, isolation and Natural selection.

Mutation theory

- Hugo de Vries put forth the Mutation theory. Mutations are sudden random changes that occur in an organism that is not heritable. De Vries carried out his experiments in the Evening Primrose plant (Oenothera lamarckiana) and observed variations in them due to mutation.

- According to de Vries, sudden and large variations were responsible for the origin of new species whereas Lamarck and Darwin believed in gradual accumulation of all variations as the causative factors in the origin of new species.

Salient features of Mutation Theory

- Mutations or discontinuous variation are transmitted to other generations.

- In naturally breeding populations, mutations occur from time to time.

- There are no intermediate forms, as they are fully fledged.

- They are strictly subjected to natural selection.

Modern synthetic theory

- Sewell Wright, Fisher, Mayer, Huxley, Dobzhansky, Simpson and Haeckel explained Natural Selection in the light of Post-Darwinian discoveries. According to this theory gene mutations, chromosomal mutations, genetic recombinations, natural selection and reproductive isolation are the five basic factors involved in the process of organic evolution.

- **Gene mutation** refers to the changes in the structure of the gene. It is also called gene/ point mutation. It alters the phenotype of an organism and produces variations in their offspring.

- **Chromosomal mutation** refers to the changes in the structure of chromosomes due to deletion, addition, duplication, inversion or translocation. This too alters the phenotype of an organism and produces variations in their offspring.

- **Genetic recombination** is due to crossing over of genes during meiosis. This brings about genetic variations in the individuals of the same species and leads to heritable variations.

- **Natural selection** does not produce any genetic variations but once such variations occur it favours some genetic changes while rejecting others (driving force of evolution).

- **Reproductive isolation** helps in preventing interbreeding between related organisms.

Evolution by anthropogenic sources
Natural Selection (Industrial melanism)

- Natural selection can be explained clearly through industrial melanism. Industrial melanism is a classical case of Natural selection exhibited by the peppered moth, Bistonbetularia. These were available in two colours, white and black. Before industrialization peppered moth both white and black coloured were common in England. Pre-industrialization witnessed white coloured background of the wall of the buildings hence the white coloured moths escaped from their predators. Post industrialization, the tree trunks became dark due to smoke and soot let out from the industries. The black moths camouflaged on the dark bark of the trees and the white moths were easily identified by their predators. Hence the dark coloured moth population was selected and their number increased when compared to the white moths. Nature offered positive selection pressure to the black coloured moths. The above proof shows that in a population, organisms that can adapt will survive and produce more progenies resulting in increase in population through natural selection.

- Artificial selection is a byproduct of human exploitation of forests, oceans and fisheries or the use of pesticides, herbicides or drugs. For hundreds of years humans have selected various types of dogs, all of which are variants of the single species of dog. If human beings can produce new varieties in short period, then "nature" with its vast resources and long duration can easily produce new species by selection.

Adaptive Radiation

- The evolutionary process which produces new species diverged from a single ancestral form becomes adapted to newly invaded habitats is called adaptive radiation. Adaptive radiations are best exemplified in closely related groups that have evolved in relatively short time. Darwin's finches and Australian marsupials are best examples for adaptive radiation. When more than one adaptive radiation occurs in an isolated geographical area, having the same structural and functional similarity it is due to convergent evolution.

Darwin's finches

- Their common ancestor arrived on the Galapagos about 2 million years ago. During that time, Darwin's finches have evolved into 14 recognized species differing in body size, beak shape and feeding behavior. Changes in the size and form of the beak have enabled different species to utilize different food resources such as insects, seeds, nectar from cactus flowers and blood from iguanas, all driven by Natural selection. represents some of the finches observed by Darwin. Genetic variation in the ALX1 gene in the DNA of Darwin finches is associated with variation in the beak shape. Mild mutation in the ALX1 gene leads to phenotypic change in the shape of the beak of the Darwin finches.

- Marsupials in Australia and placental mammals in North America are two subclasses of mammals they have adapted in similar way to a particular food resource, locomotory skill or climate. They were separated from the common ancestor more than 100 million year ago and each lineage continued to evolve independently. Despite temporal and geographical separation, marsupials in Australia and placental mammals in North America have produced varieties of species living in similar habitats with similar ways of life. Their overall resemblance in shape, locomotory mode, feeding and foraging are superimposed upon different modes of reproduction. This feature reflects their distinctive evolutionary relationships.

- Over 200 species of marsupials live in Australia along with many fewer species of placental mammals. The marsupials have undergone adaptive

radiation to occupy the diverse habitats in Australia, just as the placental mammals have radiated across North America.

Mechanism of evolution

- Microevolution (evolution on a small scale) refers to the changes in allele frequencies within a population. Allele frequencies in a population may change due to four fundamental forces of evolution such as natural selection, genetic drift, mutation and gene flow.

Natural selection

- It occurs when one allele (or combination of alleles of differences) makes an organism more or less fit to survive and reproduce in a given environment. If an allele reduces fitness, its frequencies tend to drop from one generation to the next.

- The evolutionary path of a given gene i.e., how its allele's change in frequency in the population across generation, may result from several evolutionary mechanisms acting at once. For example, one gene's allele frequencies might be modified by both gene flow and genetic drift, for another gene, mutation may produce a new allele, that is favoured by natural selection.

Selection

- There are mainly three types of natural selection

Stabilising Selection (centipetal selection):

- This type of selection operates in a stable environment. The organisms with average phenotypes survive whereas the extreme individuals from both the ends are eliminated. There is no speciation but the phenotypic stability is maintained within the population over generation. For example, measurements of sparrows that survived the storm clustered around the mean, and the sparrows that failed to survive the storm clustered around the extremes of the variation showing stabilizing selection.

Directional Selection:

- The environment which undergoes gradual change is subjected to directional selection. This type of selection removes the individuals from one end towards the other end of phenotypic distribution. For example, size differences between male and female sparrows. Both male and female look alike externally but differ in body weight. Females show directional selection in relation to body weight.

Disruptive Selection (centrifugal selection):

- When homogenous environment changes into heterogenous environment this type of selection is operational. The organisms of both the extreme phenotypes are selected whereas individuals with average phenotype are eliminated. This results in splitting of the population into sub population/species. This is a rare form of selection but leads to formation of two or more different species. It is also called adaptive radiation. E.g. Darwin's finches-beak size in relation to seed size inhabiting Galapagos islands.

- Group selection and sexual selection are other types of selection. The two major group selections are Altrusim and Kin selection.

Gene flow

- Movement of genes through gametes or movement of individuals in (immigration) and out (emigration) of a population is referred to as gene flow. Organisms and gametes that enter the population may have new alleles or may bring in existing alleles but in different proportions than those already in the population. Gene flow can be a strong agent of evolution.

Genetic drift / Sewall Wright Effect

- Genetic drift is a mechanism of evolution in which allele frequencies of a population change over generation due to chance (sampling error). Genetic drift occurs in all population sizes, but its effects are strong in a small population . It may result in a loss of some alleles (including beneficial ones) and fixation of other alleles. Genetic drift can have major effects, when the population is reduced in size by natural disaster due to bottle neck effect or when a small group of population splits from the main population to form a new colony due to founder's effect.

Origin and Evolution of Man

- Mammals evolved in the early Jurassic period, about 210 million years ago. Hominid evolution occurred in Asia and Africa. Hominids proved that human beings are superior to other animals and efficient in making tools and culture. The earliest fossils of the prehistoric man like Ramapithecus and Sivapithecus lived some 14 mya and were derived from ape like Dryopithecus. Dryopithecus and Ramapithecus were hairy and walked like gorillas and chimpanzees. Australopithecus lived in East African grasslands about 5 mya and was called the Australian ape man. He was about 1.5 meters tall with bipedal locomotion, omnivorous, semi erect, and lived in caves. Low forehead, brow ridges over the eyes, protruding face, lack of chin, low brain capacity of about 350 – 450 cc, human like dentition, lumbar curve in the vertebral column were his distinguishing features. Homo habilis lived about 2 mya. Their brain capacity was between 650 – 800cc, and was probably vegetarian. They had bipedal locomotion and used tools made of chipped stones.

- Homo erectus the first human like being was around 1.7 mya and was much closer to human in looks, skull was flatter and thicker than the modern man and had a large brain capacity of around 900 cc. Homo erectus probably ate meat.

- Homo ergaster and Homo erectus were the first to leave Africa. Neanderthal human was found in Neander Valley, Germany with a brain size of 1400 cc and lived between 34,000 - 1,00,000 years ago. They differ from the modern human in having semierect posture, flat cranium, sloping forehead, thin large orbits, heavy brow ridges, protruding jaws and no chin. They used animal hides to protect their bodies, knew the use of fire and buried their dead. They did not practice agriculture and animal domestication. Cro-Magnon was one of the most talked forms of modern human found from the rocks of Cro-Magnon, France and is considered as the ancestor of modern Europeans. They were not only adapted to various environmental conditions, but were also known for their cave paintings, figures on floors and walls.

- Homo sapiens or modern human arose in Africa some 25,000 years ago and moved to other continents and developed into distinct races. They had a

brain capacity of 1300 – 1600 cc. They started cultivating crops and domesticating animals.

Isolating Mechanism

- Isolation is the separation of the members of a single population into sub populations so that genetic integrity of the subpopulation can be maintained. Closely related species living in the same area do not breed together; they are prevented by isolating barriers. An isolating barrier is any evolved character of the two species that stops them from interbreeding. Several kinds of isolating barriers are distinguished. The most important distinction is Prezygotic and post zygotic isolation. Prezygotic mechanisms include those which prevent two species from coming into contact. This includes ecological, seasonal, ethological and morphological. Post zygotic mechanisms are those which act after fertilization that include hybrid sterility, hybrid inviability and hybrid breakdown.

- **Ecological isolation or habitat isolation** – the members of the same population may be separated from one another by a differences in their habitat. For example Rana areolata occupies burrows dug by mammals and tortoises during the day and breeds in grassy shallow ponds whereas Rana grylio breeds in deep waters. Due to the difference in their habitat the two species are able to maintain their respective species identities.

- **Seasonal isolation** – In this type ofisolation, difference in the breedingseasons prevents interbreeding. E.g.Toad, Bufo americanus breeds muchearly in the spring; whereas Bufofowleri breeds very late in the season.They are able to maintain their speciesidentity because of the differences in thebreeding seasons

- **Sexual or ethological isolation/Behavioural isolation** – Preventsmating due to the difference in theirsexual behavior. The species are notseparated from one another either intime or in space. The mating calls oftwo closely related species of frogs,Hyla versicolor (grey tree frog) andHylafemoralis (pine wood tree frog) aredifferent which prevents interbreeding.

- **Morphological isolation or mechanical isolation** – This type of isolation isdue to the differences in their externalgenitalia that is seen in two differentspecies. The size difference between thetoad species Bufo quercicus and Bufovalliceps, prevents their interbreeding.

- **Physiological isolation –**Thoughmating may occur, the gametes areprevented from fertilization due tomechanical or physiological factors.E.g. The sperms of Drosophila virilissurvive only for about a day whenintroduced into the sperm receptacle ofDrosophila American a while the spermsof Drosophila American a live for a longertime.

- **Cytological isolation** – Fertilizationdoes not take place due to thedifferences in the chromosome numbersbetween the two species, the bull frogRana catesbiana and gopher frog Ranaareolata

- **Hybrid inviability** – In this type, thesperm enters the egg, fertilization occursand the embryo develops into the adultbut it dies before reaching maturity.In certain fishes, frogs, beetles, even iffertilization takes place between twospecies, due to genetic incompatibilitythey do not leave any surviving offspring.

- **Hybrid sterility** – In this type,hybrids are formed due to inter specificcrosses but they are sterile due to thefailure of the chromosomes to segregatenormally during meiosis, example Mule(inter specific cross between a horse anda donkey).

- **Hybrid breakdown** – F1 Hybrids areviable and fertile, but F2 hybrids may beinviable or sterile.

Speciation

- The process by which one species evolves into one or more different species is called speciation. A.E. Emerson defines species as a 'genetically distinctive, reproductively isolated natural population'. Speciation is a fundamental process in evolution. Evolution of a new species in a single lineage is called an agenesis / phyletic speciation. If one species diverges to become two or more species it is cladogenesisor divergent evolution.

Sympatric speciation/Reproductive isolation

- It is a mode of speciation through which new species form from a single ancestral species while both species continue to inhabit the same geographical region. Two or more species are involved. New species formed due to genetic modification in the ancestor that is naturally selected can no longer breed with the parent population. Sexual isolation is

strongest. Phenotypic plasticity has emerged as potentially important first step in speciation initiated within an isolated population.

Phenotypic plasicity is the ability of single genotype to produce more than one phenotype. When this plasticity is expressed seasonally in planktons, it is referred to as cyclomorphosis.

Allopatric speciation/ Geographical speciation

- It is a mode of speciation that occurs when biological populations of similar species become isolated from each other that prevents gene flow. One species becomes two species due to geographical barriers hence new species is evolved e.g. Darwin's finches. The barriers are land separation, migration or mountain formation. When barriers occur between species, change in ecological conditions and environment leads to adaptations that produce differences. If there are no adaptations, they will not survive. Sexual isolation is weakest.

- A well studied example is the adaptation of Apple maggots that feed on apples in North America. When the apple trees were imported to North America, Apple maggot flies (Rhagoletispomonella) a parasitic insect that normally laid its eggs in the fruit of wild hawthorns until one subset of population began to lay its eggs in the fruit of domesticated apple trees (Malus domestica) that grew in the same area. This small group of apple maggot flies selected a different host species from the rest of the population and its offsprings became accustomed to domesticated apples.

Extinction of Animals
Extinction

- Extinction was common if not inevitable because species could not always adapt to large or rapid environmental changes. Theimpact of extinction can conveniently be considered at three levels.

- **Species extinction** eliminates an entire species, by an environmental event (flood etc.,) or by biological event (disease or non availability of food resource half or more).

- **Mass extinction** eliminates half or more species in a region or ecosystem, as might occur following a volcanic eruption. Five major mass extinction that

occurred since the Cambrian period. This mass extinction is often referred to as K-T extinction

- **Global extinction** eliminates most of the species on a large scale or larger taxonomic groups in the continent or the Earth. Snow ball Earth and extinction following elevation in CO2 levels are example. Extinction events opens up new habitats and so can facilitate the radiation of organisms that survived the mass extinction.

UNIT- 10 - Application of Bio Technology

- Geneticengineering involves the manipulation of DNA and naturally occurring processes such as protein synthesis for a wide range of applications including the production of therapeutically important proteins. This also involves extracting a gene from one organism and transferring it to the DNA of another organism, of the same or another species. The DNA produced in this way is referred to as recombinant DNA (rDNA) and this technique asrecombinant DNA technology. All these are part of the broad field biotechnology which can be definedas the applications of scientific and engineering principles to the processing of material by biologicalagents to provide goods and services.

- Biotechnology is an umbrella term that covers various techniques for using the properties of living things to make products or provide services. The term biotechnology was first used before 20th century for such traditional activities as making idli, dosa, dairy products, bread or wine, but none of these would be considered biotechnology in the modern sense.

Gene Therapy

If a person is born with a hereditary disease, can a corrective therapy be given for such disease? Yes, this can be done by a process known as gene therapy. This process involves the transfer of a normal gene into a person's cells that carries one or more mutant alleles. Expression of normal gene in the person results in a functional gene product whose action produces a normal phenotype. Delivery of the normal gene is accomplished by using a vector. The main thrust of gene therapy has been directed at correcting single gene mutations as in cystic fibrosis and haemophilia. At present most genetic diseases have no effective treatment and so gene therapy could offer hope for many people. There are two strategies involved in gene therapy namely; Gene augmentationtherapy which involves insertion of DNA into the genome to replace the missing gene product and Gene inhibition therapy which involves insertion of the anti sense gene which inhibits the expression of the dominant gene The two approaches to achieve gene therapy are somatic cell and germ line gene therapy.

Diffentiation between somatic cell gene therapy and germ line gene therapy

Somatic Cell Gene Therapy	Germ Line Gene Therapy

Therapeutic genes transferred into the somatic cells.	Therapeutic genes transferred into the germ cells.
Introduction of genes into bone marrow cells, blood cells, skin cells etc.,	Genes introduced into eggs and sperms.
Will not be inherited in later generations.	Heritable and passed on to later generations.

The first clinical gene therapy was given in 1990 by French Anderson to a four year oldgirl with adenosine deaminase (ADA) deficiency. ADA deficiency or SCID (Severe combined immunodeficiency) is an autosomal recessive metabolic disorder. It is caused by the deletion or dysfunction of the gene coding for ADA enzyme. In these patients the nonfunctioning T-Lymphocytes cannot elicit immune responses against invading pathogens.The right approach for SCID treatment would be to give the patient a functioning ADA which breaks down toxic biological products.

In some children ADA deficiency could be cured by bone marrow transplantation, where defective immune cells could be replaced with healthy immune cells from a donor. In some patients it can be treated by enzyme replacement therapy, in which functional ADA is injected into the patient.

During gene therapy the lymphocytes from the blood of the patient are removed and grown in a nutrient culture medium. A healthy and functional human gene, ADA cDNA encoding this enzyme is introduced into the lymphocytes using a retrovirus. The genetically engineered lymphocytes are subsequently returned to the patient. Since these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. The disease could be cured permanently if the gene for ADA isolated from bone marrow cells are introduced into the cells of the early embryonic stages.

- Somatic cell therapy involves the insertion ofa fully functional and expressible gene into atarget somatic cell to correct a genetic diseasepermanently whereas Germline gene therapyinvolves the introduction of DNA into germ cellswhich is passed on to the successive generations.

- Gene therapy involves isolation of a specificgene and making its copies and inserting theminto target cells to make the desired proteins.It is absolutely

essential for gene therapists toensure that the gene is harmless to the patientand it is appropriately expressed and that thebody's immune system does not react to theforeign proteins produced by the new genes.

Stem Cell Therapy

- Stem cells are undifferentiated cells found in most of the multi cellular animals. These cells maintain their undifferentiated state even after undergoing numerous mitotic divisions.

- Stem cell research has the potential to revolutionize the future of medicine with the ability to regenerate damaged and diseased organs. Stem cells are capable of self renewal and exhibit 'cellular potency'. Stem cells can differentiate into all types of cells that are derived from any of the three germ layers ectoderm, endoderm and mesoderm.

- In mammals there are two main types of stem cells – embryonic stem cells (ES cells) and adult stem cells. ES cells are pluripotent and can produce the three primary germ layers ectoderm, mesoderm and endoderm. Embryonic stem cells are multipotent stem cells that can differentiate into a number of types of cells (Fig. 10.5). ES cells are isolated from the epiblast tissue of the inner cell mass of a blastocyst. When stimulated ES can develop into more than 200 cells types of the adult body. ES cells are immortal i.e., they can proliferate in a sterile culture medium and maintain their undifferentiated state.

- Adult stem cells are found in various tissues of children as well as adults. An adult stem cell or somatic stem cell can divide and create another cell similar to it. Most of the adult stem cells are multipotent and can act as a repair system of the body, replenishing adult tissues.The red bone marrow is a rich source of adult stem cells.

- The most important and potential application of human stem cells is the generation of cells and tissues that could be used for cell based therapies. Human stem cells could be used to test new drugs.

Totipotency (Toti-total) is the ability of a single cell to divide and produce all of the differentiated cells in an organism.

Pluripotency (Pluri-several) refers to a stem cell that has the potential to differentiate into any of the three germ layers-ectoderm, endoderm and

mesoderm.

Multipotency (multi-Many) refers to the stem cells that can differentiate into various types of cells that are related. For example blood stem cells can differentiate into lymphocytes, monocytes , neutrophils etc.,

Oligopotency (Oligo-Few) refers to stem cells that can differentiate into few cell types. For example lymphoid or myeloid stem cells can differentiate into B and T cells but not RBC.

Unipotency (Uni- Single) refers to the ability of the stem cells to differentiate into only one cell type.

Stem Cell Banks

- Stem cell banking is the extraction, processing and storage of stem cells, so that they may be used for treatment in the future, when required. Amniotic cell bank is a facility that stores stem cells derived from amniotic fluid for future use. Stem cells are stored in banks specifically for use by the individual from whom such cells have been collected and the banking costs are paid. Cord Blood Banking is the extraction of stem cells from the umbilical cord during childbirth. While the umbilical cord and cord blood are the most popular sources of stem cells, the placenta, amniotic sac and amniotic fluid are also rich sources in terms of both quantity and quality.

Molecular Diagnostics

- Early diagnosis of infectious diseases or inherent genetic defects is essential for appropriate treatment. Early detection of the disease is not possible using conventional diagnostic methods like microscopic examinations, serum analysis and urine analysis.These laboratory techniques are indirect and not always specific. Scientists are continuously searching for specific, sensitive and simple diagnostic techniques for diagnosis of diseases. Recombinant DNA technology, Polymerase Chain Reactions (PCR) and Enzyme Linked Immunosorbent Assay (ELISA) are some of the techniques that are reliable and help in early diagnosis. Presence of pathogens like virus, bacteria, etc., is detected only when the pathogen produces symptoms in the patient. By the time the symptoms appear concentration of pathogen becomes very high in the body. However very low concentration of a bacteria or a virus, even when the symptoms of the disease does not appear, can be detected by amplification of their nucleic acid.

ELISA [Enzyme Linked Immunosorbent Assay]

- ELISA is a biochemical procedure discovered by Eva Engvall and Peter Perlmanin (1971) to detect the presence of specific antibodies or antigens in a sample of serum, urine, etc., It is a very important diagnostic tool to determine if a person is HIV positive or negative. ELISA is a tool for determining serum antibody concentrations (such as the antibodies produced in a person infected by pathogens such as HIV) and also for detecting the presence of specific antigens and hormones such as human chorionic gonadotropins.

- During diagnosis the sample suspected to contain the antigen is immobilized on the surface of an ELISA plate. The antibody specific to this antigen is added and allowed to react with the immobilized antigen. The anti-antibody is linked to an appropriate enzyme like peroxidase. The unreacted anti-antbody is washed away and the substrate of the enzyme (hydrogen peroxidase) is added with certain reagents such as 4-chloronaphthol. The activity of the enzyme yields a coloured product indicating the presence of the antigen. The intensity of the colour is directly proportional to the amount of the antigen. ELISA is highly sensitive and can detect antigens in the range of a nanogram.

- There are four kinds of ELISA namely, Direct ELISA, Indirect ELISA, sandwich ELISA and competitive ELISA. It is a highly sensitive and specific method used for diagnosis. ELISA possesses the added advantages of not requiring radioisotopes or a radiation counting apparatus.

PCR (Polymerase Chain Reaction)

- The polymerase chain reaction (PCR) is an invitro amplification technique used for synthesising multiple identical copies (billions) of DNA of interest. The technique was developed by Kary Mullis (Nobel laureate, 1993) in the year 1983.

- Denaturation, renaturation or primer annealing and synthesis or primer extension, are the three steps involved in PCR. The double stranded DNA of interest is denatured to separate into two individual strands by high temperature . This is called denaturation. Each strand is allowed to

hybridize with a primer (renaturation or primer annealing). The primer template is used to synthesize DNA by using Taq – DNA polymerase.

- During denaturation the reaction mixture is heated to 950 C for a short time to denature the target DNA into single strands that will act as a template for DNA synthesis. Annealing is done by rapid cooling of the mixture, allowing the primers to bind to the sequences on each of the two strands flanking the target DNA. During primer extension or synthesis the temperature of the mixture is increased to 750C for a sufficient period of time to allow Taq DNA polymerase to extend each primer by copying the single stranded template. At the end of incubation both single template strands will be made partially double stranded. The new strand of each double stranded DNA extends to a variable distance downstream. These steps are repeated again and again to generate multiple forms of the desired DNA. This process is also called DNA amplification.

- The PCR technique can also be used for amplifications of RNA in which case it is referred to as reverse transcription PCR (RT-PCR). In this process the RNA molecules (mRNA) must be converted to complementary DNA by the enzyme reverse transcriptase. The cDNA then serves as the template for PCR.

PCR In Clinical Diagnosis

- The specificity and sensitivity of PCR is useful for the diagnosis of inherited disorders (genetic diseases), viral diseases, bacterial diseases, etc., The diagnosis and treatment of a particular disease often requires identifying a particular pathogen. Traditional methods of identification involve culturing these organisms from clinical specimens and performing metabolic and other tests to identify them. The concept behind PCR based diagnosis of infectious diseases is simple – if the pathogen is present in a clinical specimen its DNA will be present.

Polymerase chain reaction

- Its DNA has unique sequences that can be detected by PCR, often using the clinical specimen (for example, blood, stool, spinal fluid, or sputum) in the PCR mixture. PCR is also employed in the prenatal diagnosis of inherited diseases by using chorionic villi samples or cells from amniocentesis. Diseases like sickle cell anemia, β-thalassemia and phenylketonuria can be detected by PCR in these samples. cDNA from PCR is a valuable tool for

diagnosis and monitoring retroviral infections – e.g., Tuberculosis by Mycobacterium tuberculosis.

- Several virally induced cancers, like cervical cancer caused by Papilloma virus can be detected by PCR. Sex of human beings and live stocks, embryos fertilized invitro can be determined by PCR by using primers and DNA probes specific for sex chromosomes. PCR technique is also used to detect sex-linked disorders in fertilized embryos.

Applications of PCR

- The differences in the genomes of two different organisms can be studied by PCR. PCR is very important in the study of evolutions, more specifically phylogenetics.

- As a technique which can amplify even minute quantities of DNA from any source, like hair, mummified tissues, bones or any fossilized materials.

- PCR technique can also be used in the field of forensic medicine . A single molecule of DNA from blood stains, hair, semen of an individual is adequate for amplification by PCR. The amplified DNA is used to develop DNA fingerprint which is used as an important tool in forensic science.Thus, PCR is very useful for identification of criminals. PCR is also used in amplification of specific DNA segment to be used in gene therapy.

Transgenic Animals

- In early days selective breeding methods were carried out to improve the genetic characteristics of live stock and other domestic animals. With the advent of modern biotechnology it is possible to carry out manipulations at the genetic level to get the desired traits in animals. Transgenesis is the process of introduction of extra (foreign/exogenous) DNA into the genome of the animals to create and maintain stable heritable characters. The foreign DNA that is introduced is called the transgene and the animals that are produced by DNA manipulations are called transgenic animals or the genetically engineered or genetically modified organisms.

The various steps involved in the production of transgenic organisms are

- Identification and separation of desired gene.

- Selection of a vector (generally a virus) or direct transmission.

- Combining the desired gene with the vector.

- Introduction of transferred vector into cells, tissues, embryo or mature individual.

- Demonstration of integration and expression of foreign gene in transgenic tissue or animals. Transgenic animals such as mice, rat, rabbit, pig, cow, goat, sheep and fish have been produced.

Uses Of Transgenesis

- Transgenesis is a powerful tool to study gene expression and developmental processes in higher organisms.

- Transgenesis helps in the improvement of genetic characters in animals.Transgenic animals serve as good models for understanding human diseases which help in the investigation of new treatments for diseases.Transgenic models exist for many human diseases such as cancer, Alzheimer's, cystic fibrosis, rheumatoid arthritis and sickle cell anemia.

- Transgenic animals are used to produce proteins which are important for medical and pharmaceutical applications.

- Transgenic mice are used for testing the safety of vaccines.

- Transgenic animals are used for testing toxicity in animals that carry genes which make them sensitive to toxic substances than non-transgenic animals exposed to toxic substances and their effects are studied.

- Transgenesis is important for improving the quality and quantity of milk, meat, eggs and wool production in addition to testing drug resistance.

Biological products and their uses

- A biological product is a substance derived from a living organism and used for the prevention or treatment of disease. These products include antitoxins, bacterial and viral vaccines, blood products and hormone extracts. These products may be produced through biotechnology in a living system, such as a microorganism, plant cell or animal cell, and are often more difficult to characterize than small molecule drugs. Through recombinant DNA technology it is possible to produce these biological products on demand. There are many types of biological products approved for use -they are, therapeutic proteins, monoclonal antibodies and vaccines. Health care and pharmaceutical industries have been revolutionised by biotechnological proteins. Hormones and antibodies are produced commercially, primarily for the medical industry. Recombinant hormones like Insulin, Human growth hormone, Recombinant vaccines and recombinant proteins like human alpha lactalbumin are available today.

- Animals are used as bioreactors to produce desirable proteins. Antibodies are substances that react against the disease causing antigens and these can be produced using transgenic animals as bioreactors. Monoclonal antibodies, which are used to treat cancer, heart disease and transplant rejection are produced by this technology. Natural protein adhesives are non toxic, biodegradable and rarely trigger an immune response, hence could be used to reattach tendons and tissues, fill cavities in teeth, and repair broken bones.

Animal Cloning

- Cloning is the process of producing genetically identical individuals of an organism either naturally or artificially. In nature many organisms produce clones through asexual reproduction.

- Cloning in biotechnology refers to the process of creating copies of organisms or copies of cells or DNA fragments (molecular cloning).

- Dolly was the first mammal (Sheep) clone developed by Ian Wilmut and Campbell in 1997. Dolly, the transgenic clone was developed by the nuclear transfer technique and the phenomenon of totipotency. Totipotency refers to the potential of a cell to develop different cells, tissues, organs and finally an organism.

- The mammary gland udder cells (somatic cells) from a donor sheep (ewe) were isolated and subjected to starvation for 5 days. The udder cells could not undergo normal growth cycle, entered a dormant stage and became totipotent. An ovum (egg cell) was taken from another sheep (ewe) and its nucleus was removed to form an enucleated ovum. The dormant mammary gland cell/udder cell and the enucleated ovum were fused. The outer membrane of the mammary cell was ruptured allowing the ovum to envelope the nucleus. The fused cell was implanted into another ewe which served as a surrogate mother. Five months later dolly was born. Dolly was the first animal to be cloned from a differentiated somatic cell taken from an adult animal without the process of fertilization

Advantages and Disadvantages Of Cloning Animals:

- Offers benefits for clinical trials and medical research. It can help in the production of proteins and drugs in the field of medicine.
- Aids stem cell research.
- Animal cloning could help to save endangered species.
- Animal and human activists see it as a threat to biodiversity saying that this alters evolution which will have an impact on populations and the ecosystem.
- The process is tedious and very expensive.
- It can cause animals to suffer.
- Reports show that animal surrogates were manifesting adverse outcomes and cloned animals were affected with disease and have high mortality rate.
- It might compromise human health through consumption of cloned animal meat.
- Cloned animals age faster than normal animals and are less healthy than the parent organism as discovered in Dolly.
- Cloning can lead to occurrence of genetic disorders in animals.

- More than 90% of cloning attempts fail to produce a viable offspring.

Ian Wilmut and Campbell removed 277 cells from the udder of an adult sheep and fused those cells with 277 unfertilised egg cells from which the nuclear material was removed. After culturing the resulting embryos for 6 days , they implanted 29 embryos into the surrogate mother's womb and only one Dolly was produced.

A gene 'knock out' is a genetically engineered organism that carries one or more genes in its chromosomes that have been made inoperative.

Ethical Issues

- Biotechnology has given to the society cheap drugs, better friuts and vegetables, pest resistant crops, indigenious cure to diseases and lot of controversy. This is mainly because the major part of the modern biotechnology deals with genetic manipulations. People fear that these genetic manipulations may lead to unknown consequences. The major apprehension of recombinant DNA technology is that unique microorganisms either inadvertently or deliberately for the purpose of war may be developed that could cause epidemics or environmentalcatastrophies. Although many are concerned about the possible risk of genetic engineering, the risks are in fact slight and the potential benefits are substantial.

Regulations in Biotechnology

- Regulations apply to the production, sale and use of biotech products and genetically modified organisms. GMOs are carefully tested and documented before the products are available. GMOs should be labelled and used according to instructions. These regulations are designed to protect the people, living organisms and the environment. The Biotechnology Regulatory Authority of India (BRAI) is a proposed regulatory body in India for uses of biotechnology products including GMOs. The Genetic Engineering Approval Committee (GEAC), a body under the Ministry of Environment, forests and climate change (India) is responsible for approval of genetically engineered products in India. If the bill is passed the responsibility will be taken over by the Environmental Appraisal Panel, a subdivision of the BRAI. The bill also proposes setting up an inter ministerial governing body to oversee the performance of BRAI

and a National Biotechnology Advisory Council of stakeholders to provide feedback on the use of, import and manufacture of biotechnology products and organisms in the society. The regulatory body is an autonomous and statutory agency to regulate the research, transport, import and manufacture of biotechnology products and organisms.

- GEAC is assisted by the State Biotechnology Co-ordination Committee (SBCC) and District Level committee (DLC). The most important committees are The Institutional Biosafety Committee (IBSC), responsible for the local implementation of guidelines; Review Committee on Genetic Manipulation (RCGM) is responsible for issuing permits and the GEAC is responsible for monitoring the large scale and commercial use of transgenic materials.

- The biotechnology industry is governed by different enactments depending on their relevance / applicability on a case to case basis. "Recombinant DNA safety guidelines, 1990" were released by the Department of Biotechnology (DBT) which cover areas of research involving genetically engineered organisms and these guidelines were further revised in 1994.

- RCGM under the DBT comprises representatives of DBT, Indian Council for Medical Research, Indian Council for Agricultural research and Council for Scientific and Industrial Research.

Possible threats of Genetically Modified Organisms

- Genetically Modified Organisms (GMOs) also called Genetically Engineered organisms (GEOs) are created to play a role in agriculture, forestry, aquaculture, bioremediation and environmental management in developed and developing countries. However, deliberate or inadvertent release of GMOs into the environment could have negative ecological effects under certain circumstances.

The possible risks of GMOs

- Creating new or more vigorous pests and pathogens. Worsening the effects of existing pests through hybridization with related transgenic organisms.

    - Harming non-target species such as soil organisms, non-pest insects, birds and other animals.

- Disrupting biotic communities including agro ecosystems.
- Irreparable loss or changes in species diversity or genetic diversity within species.

- Creating risks for human health.

- The release of GMOs into the environment could also have far reaching consequences. This is because the living GMOs proliferate, persist,disperse and sometimes may transfer their DNA into other organisms. GEOs could also displace the existing organism and create new species which may cause severe environmental damage. Due to these risks the regulatory authorities are very careful in permitting the field trials of GMOs into the environment.

Biosafety Guidelines

- Due to the growing concerns arising from Genetically Modified Organisms (GMOs) throughout the globe the WHO has built an informal working group on biosafety in 1991. This group prepared the 'voluntary code for the release of organisms into the environment'. ICGEB (International Centre for Genetic Engineering and Biotechnology) has played a significant role in issues related to biosafety and the environmentally sustainable use of biotechnology. The main 'topic of concern' related to the release of GMO's are risks for human health, environment, and agriculture which is found on the website of ICGEB.

- In India, DBT has evolved 'rDNA safety guidelines' to exercise powers conferred through the Environmental Protection Act 1986 for the manufacture, use, import, export and storage of hazardous micro organisms and genetically engineered organisms, cells etc., These guidelines are implemented and monitored by the Institutional Biosafety Committees (IBSCs), the Review Committee on Genetic Manipulation (RCGM) and the Genetic Engineering Approval Committee (GEAC) of the Ministry of Environment and Forest.

Intellectual Property Rights (IPR) and Protection (IPP)

- The physical objects like household goods or land or properties of a person and the ownership and rights on these properties is protected by certain

laws operating in the country. This type of physical property is tangible; but the transformed microorganisms, plants, animals and technologies for the production of commercial products are exclusively the property of the intellectuals. The discoverer or inventor has complete rights on his property or invention. The rights of intellectuals are protected by laws framed by a country. The intellectual property is an intangible asset. Legal rights or patents provide an inventor only a temporary monopoly on the use of an invention, in return for disclosing the knowledge to the others who may use the knowledge to develop further inventions and innovations.

- The laws are formulated from time to time at national and international levels. Development of new crop varieties is also an intellectual property right. It is protected by 'plant breeders rights' (PBRs). PBRs recognize the fact that farmers and rural communities have contributed to the creation, conservation, exchange and knowledge of genetic and species utilization of genetic diversity. IPR and IPP are granted by the Government to plant breeders for producing a specific plant variety that is new and never existed before.

- IPR is protected by different ways like patents, copyrights and trade marks.

Patents

- The science of biotechnology involves the production of enormous number of commercial products of economic importance. The inventions include biotechnology products and processes. The products include living entities like micro organisms, animals, plants, cell lines, cell organelles, plasmids and genes and naturally occurring products like primary and secondary metabolites produced by living systems e.g. alcohol, antibiotics.

- The biotechnological processes involve isolation, purification, cultivation, bioconversion of novel, innovative, simple and cost effective processes, and creation of biotechnological products.

- A patent is a Government issued document that allows the person for an exclusive right to manufacture, use or sell aninvention for a defined period (usually 20 years). It is a legal document safeguarding the rights and privileges of an inventor / invention. The purpose of patenting in biotechnology ensures fair financial returns for those who have invested finances, ideas, time and hard work for an invention.

The following criteria must be satisfied for patenting:

- The invention must be novel and useful;
- The product must be inventive and reproducible;
- The patent application should provide the full description of the invention and the invention must be patentable.

The first living organism that was patented was a genetically engineered species of bacteria - Pseudomonas putida in 1980 which was genetically engineered by Ananda Mohan Chakrabarty in 1971.

12th Botany
Classical Genetics

- Genetics is the study of how living things receive common traits from previous generations. No field of science has changed the world more, in the past 50 years than genetics. The scientific and technological advances in genetics have transformed agriculture, medicine and forensic science etc.

- Genetics – The Science of heredity (Inheritance) - "Genetics" is the branch of biological science which deals with the mechanism of transmission of characters from parents to off springs. Th e term Genetics was introduced by W. Bateson in 1906.

The four major subdisciplines of genetics are

- **Transmission Genetics / Classical Genetics** – Deals with the transmission of genes from parents to off springs. The foundation of classical genetics came from the study of hereditary behaviour of seven genes by Gregor Mendel.

- **Molecular Genetics** – Deals with the structure and function of a gene at molecular level.

- **Population Genetics** – Deals with heredity in groups of individuals for traits which is determined by a few genes.

- **Quantitative Genetics** – Deals with heredity of traits in groups of individuals where the traits are governed by many genes simultaneously.

What is the reason for similarities, diff erences of appearance and skipping of generations? Genes – Functional Units of inheritance: The basic unit of heredity (biological information) which transmits biochemical, anatomical and behavioural traits from parents to off springs.

Heredity and variation

- Genetics is often described as a science which deals with heredity and variation.

- **Heredity:** Heredity is the transmission of characters from parents to offsprings.

- **Variation:** The organisms belonging to the same natural population or species that shows a difference in the characteristics is called variation. Variation is of two types (i) Discontinuous variation and (ii) Continuous variation

1. **Discontinuous Variation:**

   Within a population there are some characteristics which show a limited form of variation. Example: Style length in Primula, plant height of garden pea. In discontinuous variation, the characteristics are controlled by one or two major genes which may have two or more allelic forms. These variations are genetically determined by inheritance factors. Individuals produced by this variation show differences without any intermediate form between them and there is no overlapping between the two phenotypes. The phenotypic expression is unaffected by environmental conditions. This is also called as qualitative inheritance.

2. **Continuous Variation**:

   This variation may be due to the combining effects of environmental and genetic factors. In a population most of the characteristics exhibit a complete gradation, from one extreme to the other without any break. Inheritance of phenotype is determined by the combined effects of many genes, (polygenes) and environmental factors. This is also known as quantitative inheritance. Example: Human height and skin color.

Importance of variations

- Variations make some individuals betterfitted in the struggle for existence.
- They help the individuals to adapt themselves to the changing environment.
- It provides the genetic material for natural selection
- Variations allow breeders to improve better yield, quicker growth, increased resistance and lesser input.

- They constitute the raw materials for evolution.

Mendelism

- The contribution of Mendel to Genetics is called Mendelism. It includes all concepts brought out by Mendel through his original research on plant hybridization. Mendelian genetic concepts are basic to modern genetics. Therefore, Mendel is called as Father of Genetics.

Father of Genetics – Gregor Johann Mendel (1822 – 1884)

- The first Geneticist, Gregor Johann Mendel unraveled the mystery of heredity. He was born on 22nd July 1822 in Heinzendorf Silesia (now Hyncice, Czechoslovakia), Austria. After school education, later he studied botany, physics and mathematics at the University of Vienna.He then entered a monastery of St.Thomas at Brunn in Austria and continued his interest in plant hybridization.In 1849 Mendel got a temporary position in a school as a teacher and he performed a series of elegant experiments with pea plants in his garden. In 1856, he started his historic studies on pea plants. 1856 to 1863 was the period of Mendel's hybridization experiments on pea plants. Mendel discovered the principles of heredity by studying the inheritance of seven pairs of contrasting traits of pea plant in his garden. Mendel crossed and catalogued 24,034plants through many generations. His paper entitled "Experiments on Plant Hybrids" was presented and published in The Proceedings of the Brunn Society of Natural History in 1866. Mendel was the first systematic researcher in the field of genetics.

Mendel was successful because:

- He applied mathematics and statisticalmethods to biology and laws of probabilityto his breeding experiments.

- He followed scientific methods and keptaccurate and detailed records that includequantitative data of the outcome of hiscrosses.

- His experiments were carefully plannedand he used large samples.

- The pairs of contrasting characters whichwere controlled by factor (genes)werepresent on separate chromosomes.

- The parents selected by Mendel were purebreed lines and the purity was tested by selfcrossing the progeny for many generations.

Mendel's Experimental System – The Garden pea.
He chose pea plant because,

- It is an annual plant and has clearcontrasting characters that are controlledby a single gene separately.

- Self-fertilization occurred under normalconditions in garden pea plants. Mendelused both self-fertilization and cross-fertilization.

- The flowers are large hence emasculationand pollination are very easy forhybridization.

Mendel's experiments on pea plant

- Mendel's theory of inheritance, known as the Particulate theory, establishes the existence of minute particles or hereditary units or factors, which are now called as genes. He performed artificial pollination or cross pollination experiments with several true-breeding lines of pea plants. A true breeding lines (Pure-breeding strains) means it has undergone continuous self pollination having stable trait inheritance from parent to offspring. Matings within pure breeding lines produce offsprings having specific parental traits that are constant in inheritance and expression for many generations. Pure line breed refers to homozygosity only. Fusion of male and female gametes produced by the same individual i.e pollen and egg are derived from the same plant is known as self-fertilization. Self pollination takes place in Mendel's peas. The experimenter can remove the anthers (Emasculation) before fertilization and transfer the pollen from another variety of pea to the stigma of flowers where the anthers are removed. This results in cross-fertilization, which leads to the creation of hybrid varieties with different traits. Mendel's work on the study of the pattern of inheritance and the principles or laws formulated, now constitute the Mendelian Genetics.

Can you identify Mendel's gene for regulating white colour in peas? Let us find the molecular answer to understand the gene function. Now the genetic mystery of Mendel's white flowers is solved.
It is quite fascinating to trace the Mendel's genes. In 2010, the gene responsible

for regulating flower colour in peas were identified by an international team of researchers. It was called Pea Gene A which encodes a protein that functions as a transcription factor which is responsible for the production of anthocyanin pigment. So the flowers are purple. Pea plants with white flowers do not have anthocyanin, even though they have the gene that encodes the enzyme involved in anthocyanin synthesis.
Researchers delivered normal copies of gene A into the cells of the petals of white flowers by the gene gun method. When Gene A entered in a small percentage of cells of white flowers it is expressed in those particular cells, accumulated anthocyanin pigments and became purple.
In white flowers the gene A sequence showed a single-nucleotide change that makes the transcription factor inactive. So the mutant form of gene A do not accumulate anthocyanin and hence they are white.

- Mendel worked at the rules of inheritance and arrived at the correct mechanism before any knowledge of cellular mechanism, DNA, genes, chromosomes became available. Mendel insights and meticulous work into the mechanism of inheritance played an important role which led to the development of improved crop varieties and a revolution in crop hybridization.

- Mendel died in 1884. In 1900 the work of Mendel's experiments were rediscovered by three biologists, Hugo de Vries of Holland, Carl Correns of Germany and Erich von Tschermak of Austria.

Terminology related to Mendelism

- Mendel noticed two different expressions of a trait – Example: Tall and dwarf. Traits are expressed in different ways due to the fact that a gene can exist in alternate forms (versions) for the same trait is called alleles.

- If an individual has two identical alleles of a gene, it is called as homozygous(TT). An individual with two different alleles is called heterozygous(Tt). Mendels non-true breeding plants are heterozygous, called as hybrids

- When the gene has two alleles the dominant allele is symbolized with capital letter and the recessive with small letter. When both alleles are recessive the individual is called homozygous recessive (tt) dwarf pea plants. An individual with two dominant alleles is called homozygous

dominant (TT) tall pea plants. One dominant allele and one recessive allele (Tt) denotes non-true breeding tall pea plants heterozygous tall.

Mendelian inheritance – Mendel's Laws of Heredity

- Mendel proposed two rules based on his observations on monohybrid cross, today these rules are called laws of inheritance The first law is The Law of Dominance and the second law is The Law of Segregation. These scientific laws play an important role in the history of evolution.

The Law of Dominance:

- The characters are controlled by discrete units called factors which occur in pairs. In a dissimilar pair of factors one member of the pair is dominant and the other is recessive. This law gives an explanation to the monohybrid cross (a) the expression of only one of the parental characters in F1 generation and (b) the expression of both in the F2 generation. It also explains the proportion of 3:1 obtained at the F2.

The Law of Segregation (Law of Purity of gametes):

- Alleles do not show any blending, both characters are seen as such in the F2 generation although one of the characters is not seen in the F1 generation. During the formation of gametes, the factors or alleles of a pair separate and segregate from each other such that each gamete receives only one of the two factors. A homozygous parent produces similar gametes and a heterozygous parent produces two kinds of gametes each having one allele with equal proportion. Gametes are never hybrid.

Monohybrid cross

- Monohybrid inheritance is the inheritance of a single character i.e. plant height. It involves the inheritance of two alleles of a single gene. When the F1 generation was selfed Mendel noticed that 787 of 1064 F2 plants were tall, while 277 of 1064 were dwarf. The dwarf trait disappeared in the F1 generation only to reappear in the F2 generation. The term genotype is the genetic constitution of an individual. The term phenotype refers to the observable characteristic of an organism. In a genetic cross the genotypes and phenotypes of offspring, resulting from combining gametes during fertilization can be easily understood with the help of a diagram called Punnett's Square named after a British Geneticist Reginald C.Punnett. It is a graphical representation to calculate the probability of all possible

genotypes of offsprings in a genetic cross. The Law of Dominance and the Law of Segregation give suitable explanation to Mendel's monohybrid cross.

Reciprocal cross

- In one experiment, the tall pea plants were pollinated with the pollens from a true-breeding dwarf plants, the result was all tall plants. When the parental types were reversed, the pollen from a tall plant was used to pollinate a dwarf pea plant which gave only tall plants. The result was the same - All tall plants. Tall x Dwarf and Tall x Dwarf matings are done in both ways which are called reciprocal crosses.The results of the reciprocal crosses are the same. So it was concluded that the trait is not sex dependent. The results of Mendel's monohybrid crosses were not sex dependent.

- The gene for plant height has two alleles: Tall (T)x Dwarf (t). The phenotypic and genotypicanalysis of the crosses has been shown byChecker board method or by Forkline method.

Mendel's analytical and empirical approach

- Mendel chose two contrasting traits for each character. So it seemed logical that two distinct factors exist. In F1 the recessive trait and its factors do not disappear and they are hidden or masked only to reappear in ¼ of the F2 generation. He concluded that tall and dwarf alleles of F1 heterozygote segregate randomly into gametes. Mendel got 3:1 ratio in F2 between the dominant and recessive trait. He was the first scientist to use this type of quantitative analysis in a biological experiment. Mendel's data is concerned with the proportions of offspring.

- Mendel's analytical approach is truly an outstanding scientific achievement. His meticulous work and precisely executed breeding experiments proposed that discrete particulate units of heredity are present and they are transmitted from one generation to the other. Now they are called as genes. Mendel's experiments were well planned to determine the relationships which govern hereditary traits. This rationale is called an empirical approach. Laws that were arrived from an empirical approach is known as empirical laws.

Test cross

- Test cross is crossing an individual of unknowngenotype with a homozygous recessive.

- In Mendel's monohybrid cross all the plants are tall in F1 generation. In F2 tall and dwarf plants were in the ratio of 3:1.Mendel self pollinated dwarf F2 plants and got dwarf plants in F3 and F4 generations. So he concluded that the genotype of dwarf was homozygous (tt). The genotypes of tall plants TT or Tt from F1and F2 cannot be predicted. But how we can tell if a tall plant is homozygous or heterozygous? To determine the genotype of a tall plant Mendel crossed the plants from F2 with the homozygous recessive dwarf plant. This he called a test cross. The progenies of the test cross can be easily analysed to predict the genotype of the plant or the test organism. Thus in a typical test cross an organism (pea plants) showing dominant phenotype (whose genotype is to be determined) is crossed with the recessive parent instead of self crossing. Test cross is used to identify whether an individual is homozygous or heterozygous for dominant character.

Back Cross

- Back Cross is a cross of F1hybrid with any one of the parental genotypes . The back cross is f two type; they are dominant back cross and recessive back cross.

- It involves the cross between the F1 offspringwith either of the two parents.

- When the F1offsprings are crossed withthe dominant parents all the F2 developdominant character and no recessiveindividuals are obtained in the progeny.

- If the F1 hybrid is crossed with the recessive parent individuals of both the phenotypes appear in equal proportion and this cross is specified as test cross.

- The recessive back cross helps to identify the heterozygosity of the hybrid.

Dihybrid cross

- It is a genetic cross which involves individuals differing in two characters. Dihybrid inheritance is the inheritance of two separate genes each with two alleles.

- **Law of Independent Assortment** – When two pairs of traits are combined in a hybrid, segregation of one pair of characters is independent to the other pair of characters. Genes that are located in different chromosomes assort independently during meiosis. Many possible combinations of factors can occur in the gametes.

- Independent assortment leads to genetic diversity. If an individual produces genetically dissimilar gametes it is the consequence of independent assortment. Through independent assortment, the maternal and paternal members of all pairs were distributed to gametes, so all possible chromosomal combinations were produced leading to genetic variation. In sexually reproducing plants / organisms, due to independent assortment, genetic variation takes place which is important in the process of evolution. The Law of Segregation is concerned with alleles of one gene but the Law of Independent Assortment deals with the relationship between genes.

- The crossing of two plants differing in two pairs of contrasting traits is called dihybrid cross. In dihybrid cross, two characters (colour and shape) are considered at a time. Mendel considered the seed shape (round and wrinkled) and cotyledon colour (yellow & green) as the two characters. In seed shape round (R) is dominant over wrinkled (r) ; in cotyledon colour yellow (Y)is dominant over green (y). Hence the purebreeding round yellow parent is represented bythe genotype RRYY and the pure breeding greenwrinkled parent is represented by the genotyperryy. During gamete formation the paired genesof a character assort out independently of theother pair. During the F_1 x F_1 fertilization eachzygote with an equal probability receives oneof the four combinations from each parent.The resultant gametes thus will be geneticallydifferent and they are of the following four types:

Yellow round (YR)	9/16
Yellow wrinkled (Yr)	3/16

Green round (yR) 3/16

Green wrinkled (yr) 1/16

- These four types of gametes of F1 dihybridsunite randomly in the process of fertilization and produce sixteen types of individuals in F2 in the ratio of 9:3:3:1 as shown in the figure. Mendel's 9:3:3:1 dihybrid ratio is an ideal ratio based on the probability including segregation, independent assortment and randomfertilization. In sexually reproducing organism / plants from the garden peas to human beings, Mendel's findings laid the foundation for understanding inheritance and revolutionized the field of biology. The dihybrid cross and its result led Mendel to propose a second set of generalisations that we called Mendel's Law of independent assortment.

How does the wrinkled gene make Mendel's peas wrinkled? Find out the molecular explanation.

The protein called starch branching enzyme (SBEI) is encoded by the wild-type allele of the gene (RR) which is dominant. When the seed matures, this enzyme SBEI catalyzes the formation of highly branched starch molecules. Normal gene (R) has become interrupted by the insertion of extra piece of DNA (0.8 kb) into the gene, resulting in r allele. In the homozygous mutant form of the gene (rr) which is recessive, the activity of the enzyme SBEI is lost resulting in wrinkled peas. The wrinkled seed accumulates more sucrose and high water content. Hence the osmotic pressure inside the seed rises. As a result, the seed absorbs more water and when it matures it loses water as it dries. So it becomes wrinkled at maturation. When the seed has atleast one copy of normal dominant gene heterozygous, the dominant allele helps to synthesize starch, amylopectin an insoluble carbohydrate, with the osmotic balance which minimises the loss of water resulting in smooth structured round seed.

Trihybrid cross

- The trihybrid cross demonstrates that Mendel's laws are applicable to the inheritance of multiple traits. Mendel Laws of segregation and independent assortment are also applicable to three pairs of contrasting characteristic traits called trihybrid cross.

- A cross between homozygous parents that differ in three gene pairs (i.e. producing trihybrids) is called trihybrid cross. A self fertilizing trihybrid plant forms 8 different gametes and 64 different zygotes. In this a combination of three single pair crosses operating together.

Extensions of Mendelian Genetics

- Apart from monohybrid, dihybrid and trihybrid crosses, there are exceptions to Mendelian principles, i.e. the occurrence of different phenotypic ratios. The more complex patterns of inheritance are the extensions of Mendelian Genetics. There are examples where phenotype of the organism is the result of the interactions among genes.

- **Gene interaction –** A single phenotype is controlled by more than one set of genes, each of which has two or more alleles. This phenomenon is called Gene Interaction. Many characteristics of the organism including structural and chemical which constitute the phenotype are the result of interaction between two or more genes.

- Mendelian experiments prove that a single gene controls one character. But in the post Mendelian findings, various exception have been noticed, in which different types of interactions are possible between the genes. This gene interaction concept was introduced and explained by W. Bateson. This concept is otherwise known as Factor hypothesis or Bateson's factor hypothesis. According to Bateson's factor hypothesis, the gene interactions can be classified as

    - Intragenic gene interactions or Intra allelic or allelic interactions
    - Intergenic gene interactions or inter allelic or non-allelic interactions.

Intragenic gene interactions

- Interactions take place between the alleles of the same gene i.e., alleles at the same locus is called intragenic or intralocus gene interaction. It includes the following:

    - Incomplete dominance
    - Codominance
    - Multiple alleles
    - Pleiotropic genes are common examples for intragenic interaction.

Incomplete dominance – No blending of genes

- The German Botanist Carl Correns's (1905) Experiment - In 4 O' clock plant, Mirabilis jalapa when the pure breeding homozygous red (R1R1) parent is crossed with homozygous white (R2R2), the phenotype of the F1 hybrid is heterozygous pink (R1R2). The F1 heterozygous phenotype differs from both the parental homozygous phenotype. This cross did not exhibit the character of the dominant parent but an intermediate colour pink. When one allele is not completely dominant to another allele it shows incomplete dominance. Such allelic interaction is known as incomplete dominance. F1 generation produces intermediate phenotype pink coloured flower. When pink coloured plants of F1 generationwere interbred in F_2 both phenotypic and genotypic ratios were found to be identical as 1 : 2 : 1(1 red : 2 pink : 1 white). Genotypic ratio is 1 R_1R_1 : 2 R_1R_2 : 1 R_2R_2.From this we conclude that the alleles themselves remain discrete and unaltered proving the Mendel's Law of Segregation. The phenotypic and genotypic ratios are the same. There is no blending of genes. In the F_2 generation R_1 and R_2 genes segregate and recombine to produce red, pink and white in the ratio of 1 : 2 : 1. R_1 allele codes for an enzyme responsible for the formation of red pigment. R_2 allele codes for defective enzyme. R_1 and R_2 genotypes produce only enough red pigments to make the flower pink. Two R_1R_1 are needed for producing red flowers. Two R_2R_2 genes are needed for white flowers. If blending had taken place, the original pure traits would not have appeared and all F_2 plants would have pink flowers. It is very clear that Mendel's particulate inheritance takes place in this cross which is confirmed by the reappearanceof original phenotype in F_2.

How will you explain incomplete dominance at the molecular level?
Gene expression is explained in a quantitative way. Wild-type allele which is a functional allele when present in two copies (R1 R1) produces an functional enzyme which synthesizes red pigments. The mutant allele which is a defective allele in two copies (R2 R2) produces an enzyme which cannot synthesize necessary red pigments. The white flower is due to the mutation causing complete loss of function. The F1 intermediate phenotype heterozygote (R1R2) has one copy of the allele R1. R1 produces 50% of the functional protein resulting in half of the pigment of red flowered plant and so it is pink. The intermediate phenotype pink heterogyzote with 50% of functional protein is not enough to create the red phenotype homozygous, which makes 100% of the functional protein.

Codominance (1 : 2 : 1)

- This pattern occurs due to simultaneous (joint) expression of both alleles in the heterozygote - The phenomenon in which two alleles are both expressed in the heterozygous individual is known as codominance. Example: Red and white flowers of Camellia, inheritance of sickle cell haemoglobin, ABO blood group system in humanbeings. In humanbeings, IA and IB alleles of I gene are codominant which follows Mendels law of segregation. The codominance was demonstrated in plants with the help of electrophoresis or chromatography for protein or flavonoid substance. Example: Gossypium hirsutum and Gossypium sturtianum, their F1 hybrid (amphiploid) was tested for seed proteins by electrophoresis. Both the parents havedifferent banding patterns for their seed proteins. In hybrids, additive banding pattern was noticed. Their hybrid shows the presence of both the types of proteins similar to their parents.

- The heterozygote genotype gives rise to a phenotype distinctly different from either of the homozygous genotypes. The F1 heterozygotes produce a F2 progeny in a phenotypic and genotypic ratios of 1 : 2 : 1.

Lethal genes

- An allele which has the potential to cause the death of an organism is called a "Lethal Allele". In 1907, E. Baur reported a lethal gene in snapdragon (Antirrhinum sp.). It is an example for recessive lethality. In snapdragon there are three kinds of plants.

- Green plants with chlorophyll. (CC)

- Yellowish green plants with carotenoids arereferred to as pale green, golden or aureaplants (Cc)

- White plants without any chlorophyll. (cc)

- The genotype of the homozygous greenplants is CC. The genotype of the homozygous white plant is cc.

- The aurea plants have the genotype Cc because they are heterozygous of green and white plants. When two such aurea plants are crossed the F1

progeny has identical phenotypic and genotypic ratio of 1 : 2 : 1 (viz. 1 Green (CC) : 2 Aurea (Cc) : 1 White (cc))

- Since the white plants lack chlorophyll pigment, they will not survive. So the F2 ratio is modified into 1 : 2. In this case the homozygous recessive genotype (cc) is lethal.

- The term "lethal" is applied to those changes in the genome of an organism which produces effects severe enough to cause death. Lethality is a condition in which the death of certain genotype occurs prematurely. The fully dominant or fully recessive lethal allele kills the carrier individual only in its homozygous condition. So the F2 genotypic ratio will be 2 : 1 or 1 : 2 respectively.

Pleiotropy – A single gene affects multiple traits

- In Pleiotropy, the single gene affects multiple traits and alter the phenotype of the organism. The Pleiotropic gene influences a number of characters simultaneously and such genes are called pleiotropic gene. Mendel noticed pleiotropy while performing breeding experiment with peas (Pisum sativum). Peas with purple flowers, brown seeds and dark spot on the axils of the leaves were crossed with a variety of peas having white flowers, light coloured seeds and no spot on the axils of the leaves, the three traits for flower colour, seed colour and a leaf axil spot all were inherited together as a single unit. This is due to the pattern of inheritance where the three traits were controlled by a single gene with dominant and recessive alleles. Example: sickle cell anemia.

Intergenic gene interactions

- Interlocus interactions take place between the alleles at different loci i.e between alleles of different genes.It includes the following:

- **Dominant Epistasis –** It is a gene interaction in which two alleles of a gene at one locus interfere and suppress or mask the phenotypic expression of a different pair of alleles of another gene at another locus. The gene that suppresses or masks the phenotypic expression of a gene at another locus is known as epistatic. The gene whose expression is interfered by non-allelic genes andprevents from exhibiting its character is known as hypostatic.

When both the genes are present together, the phenotype is determined by the epistatic gene and not by the hypostatic gene.

- In the summer squash the fruit colour locus has a dominant allele 'W' for white colour and a recessive allele 'w' for coloured fruit. 'W' allele is dominant that masks the expression of any colour. In another locus hypostatic allele 'G' is for yellow fruit and its recessive allele 'g' for green fruit. In the first locus the white is dominant to colour where as in the second locus yellow is dominant to green. When the white fruit with genotype WWgg is crossed with yellow fruit with genotype wwGG, the F1 plants have white fruit and are heterozygous (WwGg). When F1 heterozygous plants are crossed they give rise to F2 with the phenotypic ratio of 12 white : 3 yellow : 1 green.

Intra –genic or allelic interaction

Gene interaction	**Example**	**F2Phenotypic ratio**
Incomplete Dominance	Flower colour in Mirabilis jalapa. Flower colour in snapdragon(Antirrhinum spp.)	1 : 2 : 1 1 : 2 : 1
Codominance	ABO Blood group system in humans	1 : 2 : 1

Inter-genic or non-allelic interaction

Epistatic interaction	**Example**	**F2 Ratio Phenotypic ratio**
Dominant epistasis	Fruit colour in summer squash	12 : 3 : 1
Recessive epistasis	Flower colour of Antirrhinumspp.	9 : 3 : 4
Duplicate genes with cumulative effect	Fruit shape in summer squash	9 : 6 : 1
Complementary genes	Flower colour in sweet peas	9 : 7
Supplementary genes	Grain colour in Maize	9 : 3 : 4
Inhibitor genes	Leaf colour in rice plants	13 : 3

Duplicate genes	Seed capsule shape (fruit shape) in shepherd's purse Bursa bursa-pastoris	15 : 1

Polygenic Inheritance in Wheat (Kernel colour)

- Polygenic inheritance - Several genes combine to affect a single trait.

- A group of genes that together determine (contribute) a characteristic of an organism is called polygenic inheritance. It gives explanations to the inheritance of continuous traits which are compatible with Mendel's Law.

- The first experiment on polygenic inheritance was demonstrated by Swedish Geneticist H. Nilsson - Ehle (1909) in wheat kernels. Kernel colour is controlled by two genes each with two alleles, one with red kernel colour was dominant to white. He crossed the two pure breeding wheat varieties dark red and a white. Dark red genotypes R1R1R2R2 and white genotypes are r1r1r2r2. In the F1 generation medium red were obtained with the genotype R1r1R2r2. F1 wheat plant produces four types of gametes R1R2, R1r2, r1R2, r1r2. The intensity of the red colour is determined by the number of R genes in the F2 generation.

- **Four R genes:** A dark red kernel colour is obtained.Three R genes: Medium - dark red kernel colour is obtained.Two R genes: Medium-red kernel colour is obtained.One R gene: Light red kernel colour is obtained.

- **Absence of R gene**:Results in White kernel colour.The R gene in an additive manner produces the red kernel colour. The number of each phenotype is plotted against the intensity of red kernel colour which produces a bell shaped curve. This represents the distribution of phenotype. Other example: Height and skin colour in humans are controlled by three pairs of genes.

Extra Chromosomal Inheritance or Extra Nuclear Inheritance (Cytoplasmic Inheritance)

- DNA is the universal genetic material. Genes located in nuclear chromosomes follow Mendelian inheritance. But certain traits are governed either by the chloroplast or mitochondrial genes. This phenomenon is

known as extra nuclear inheritance. It is a kind of Non-Mendelian inheritance. Since it involves cytoplasmic organelles such as chloroplast and mitochondrion that act as inheritance vectors, it is also called Cytoplasmic inheritance. It is based on independent, self-replicating extra chromosomal unit called plasmogene located in the cytoplasmic organelles, chloroplast and mitochondrion.

Chloroplast Inheritance

- It is found in 4 O' Clock plant (Mirabilis jalapa). In this, there are two types of variegated leaves namely dark green leaved plants and pale green leaved plants. When the pollen of dark green leaved plant (male) is transferred to the stigma of pale green leaved plant (female) and pollen of pale green leaved plant is transferred to the stigma of dark green leaved plant, the F1 generation of both the crosses must be identical as per Mendelian inheritance. But in the reciprocal cross the F1 plant differs from each other. In each cross, the F1 plant reveals the character of the plant which is used as female plant.

- This inheritance is not through nuclear gene. It is due to the chloroplast gene found in the ovum of the female plant which contributes the cytoplasm during fertilization since the male gamete contribute only the nucleus but not cytoplasm.

Mitochondrial Inheritance

- Male sterility found in pearl maize (Sorgum vulgare) is the best example for mitochondrial cytoplasmic inheritance. So it is called cytoplasmic male sterility. In this, male sterility is inherited maternally. The gene for cytoplasmic male sterility is found in the mitochondrial DNA.

- In this plant there are two types, one with normal cytoplasm (N) which is male fertile and the other one with aberrant cytoplasm (S) which is male sterile. These types also exhibit reciprocal differences as found in Mirabilis jalapa.

- Recently it has been discovered that cytoplasmic genetic male sterility is common in many plant species. This sterility is maintained by the influence of both nuclear and cytoplasmic genes. There are commonly two types of cytoplasm N (normal) and S (sterile). The genes for these are found in

mitochondrion. There are also restores of fertility (Rf) genes. Even though these genes are nuclear genes, they are distinct from genetic male sterility genes of other plants. Because the Rf genes do not have any expression of their own, unless the sterile cytoplasm is present. Rf genes are required to restore fertility in S cytoplasm which is responsible for sterility.

- So the combination of N cytoplasm with rfrf and S cytoplasm with RfRf produces plants with fertile pollens, while S cytoplasm with rfrf produces only male sterile plants.

Atavism

- Atavism is a modification of a biological structure whereby an ancestral trait reappears after having been lost through evolutionary changes in the previous generations. Evolutionary traits that have disappeared phenotypically do not necessarily disappear from an organism's DNA. The gene sequence often remains, but is inactive. Such an unused gene may remain in the genome for many generations. As long as the gene remains intact, a fault in the genetic control suppressing the gene can lead to the reappearance of that character again. Reemergence of sexual reproduction in the flowering plant Hieracium pilosella is the best example for Atavism in plants.

UNIT – 3
Chromosomal Basis of Inheritance

Chromosomal Theory of Inheritance

- G. J. Mendel (1865) studied the inheritance of well-defined characters of pea plant but for several reasons it was unrecognized till 1900. Three scientists (de Vries, Correns and Tschermak) independently rediscovered Mendel's results on the inheritance of characters. Various cytologists also observed cell division due to advancements in microscopy. This led to the discovery of structures inside nucleus. In eukaryotic cells, worm-shaped structures formed during cell division are called chromosomes (colored bodies, visualized by staining). An organism which possesses two complete basic sets of chromosomes are known as diploid. A chromosome consists of long, continuous coiled piece of DNA in which genes are arranged in linear order. Each gene has a definite position (locus) on a chromosome. These genes are hereditary units. Chromosomal theory of inheritance states that Mendelian factors (genes) have specific locus (position) on chromosomes and they carry information from one generation to the next generation.

Historical development of chromosome theory

- The important cytological findings related to the chromosome theory of inheritance are given below.

  - **Wilhelm Roux (1883)** postulated that the chromosomes of a cell are responsible for transferring heredity.

  - **Montgomery (1901)** was first to suggest occurrence of distinct pairs of chromosomes and he also concluded that maternal chromosomes pair with paternal chromosomes only during meiosis.

  - **T.Boveri (1902)** supported theidea that the chromosomes containgenetic determiners, and he waslargely responsible for developing thechromosomal theory of inheritance.

  - **W.S. Sutton (1902)**, a young Americanstudent independently recognized aparallelism (similarity) between thebehaviour of chromosomes and Mendelian factors during gamete formation.

- **Sutton and Boveri (1903)** independentlyproposed the chromosome theory of inheritance. Sutton united the knowledge of chromosomal segregation with Mendelian principles and called it chromosomal theory of inheritance.

Salient features of the Chromosomal theory of inheritance

- Somatic cells of organisms are derived from the zygote by repeated cell division (mitosis). These consist of two identical sets of chromosomes. One set is received from female parent (maternal) and the other from male parent (paternal). These two chromosomes constitute the homologous pair.

- Chromosomes retain their structural uniqueness and individuality throughout the life cycle of an organism.

- Each chromosome carries specific determiners or Mendelian factors which are now termed as genes.

- The behaviour of chromosomes during the gamete formation (meiosis) provides evidence to the fact that genes or factors are located on chromosomes.

Support for chromosomal theory of heredity

- This theory was widely discussed and controversies by scientists around the world. However, this debate has been finally cleared by the works of Thomas Hunt Morgan (1910) on the fruit fly Drosophila melanogaster (2n=8). This fruit fly completed their life cycle within two weeks. The alleles for red or white eye colour are present on the X chromosome but there is no counterpart for this gene on the Y chromosome. Thus, females have two alleles for this gene, whereas males have only one. The genetic results were completely based on meiotic behaviour of the X and Y chromosomes. Similarly, the genes for yellow body colour and miniature wings are also carried on the X chromosome. This study strongly supports the idea that genes are located on chromosomes. The linked genes connected together on sex chromosome is called sex linkage.

Comparison between gene and chromosome behaviour

- Around twentieth century cytologists established that, generally the total number of chromosomes is constant in all cells of a species. A diploid eukaryotic cell has two haploid sets of chromosomes, one set from each parent. All somatic cells of an organism carry the same genetic complement. The behaviour of chromosomes during meiosis not only explains Mendel's principles but leads to new and different approaches to study about heredity.

Mendelian factors	**Chromosomes behaviour**
Alleles of a factor occur in pair	Chromosomes occur in pairs
Similar or dissimilar alleles of a factor separate during the gamete formation	The homologous chromosomes separate during meiosis
Mendelian factors can assort independently	The paired chromosomes can separate independently during meiosis but the linked genes in the same chromosome normally do not assort independently

The important aspects to be remembered about the chromosome behaviour during cell division (meiosis) are as follows.

- The alleles of a genotype are found in the same locus of a homologous chromosome (A/a).
- In the S phase of meiotic interphase each chromosome replicates forming two copies of each allele (AA/aa), one on each chromatid.
- The homologous chromosomes segregate in anaphase I, thereby separating two different alleles (AA) and (aa).
- In anaphase II of meiosis, separation of sister chromatids of homologous chromosomes takes place. Therefore, each daughter cell (gamete) carries only a single allele (gene) of a character (A), (A), (a) and (a).

Fossil Genes: Some of thejunk DNA is made up of pseudogenes, the sequences presence in that was once working genes. They lost their ability to make proteins. They tell the story of evolution through fossilized parts.

Linkage

- The genes which determine the character of an individual are carried by the chromosomes. The genes for different characters may be present either in the same chromosome or in different chromosomes. When the genes are present in different chromosomes, they assort independently according to Mendel's Law of Independent Assortment. Biologists came across certain genetic characteristics that did not assort out independently in other organisms after Mendel's work. One such case was reported in Sweet pea (Lathyrus odoratus) by Willium Bateson and Reginald C. Punnet in 1906. They crossed one homozygous strain of sweet peas having purple flowers and long pollen grains with another homozygous strain having red flowers and round pollen grains. All the F1 progenies had purple flower and long pollen grains indicating purple flower long pollen (PL/PL) was dominant over red flower round pollen (pl/pl). When they crossed the F1 with double recessive parent (test cross) in results, F2 progenies did not exhibit in 1:1:1:1 ratio as expected with independent assortment. A greater number of F2 plants had purple flowers and long pollen or red flowers and round pollen. So they concluded that genes for purple colour and long pollen grain and the genes for red colour and round pollen grain were found close together in the same homologous pair of chromosomes. These genes do not allow themselves to be separated. So they do not assort independently. This type of tendency of genes to stay together during separation of chromosomes is called Linkage.

- Genes located close together on the same chromosome and inherited together are called linked genes. But the two genes that are sufficiently far apart on the same chromosome are called unlinked genes or syntenic genes. Such condition is known as synteny. It is to be differentiated by the value of recombination frequency. If the recombination frequency value is more than 50 % the two genes show unlinked. when the recombination frequency value is less than 50 %, they show linked. Closely located genes show strong linkage, while genes widely located show weak linkages.

Coupling and Repulsion theory

- The two dominant alleles or recessive alleles occur in the same homologous chromosomes, tend to inherit together into same gamete are called coupling or cis configuration. If dominant or recessive alleles are present on two

different, but homologous chromosomes they inherit apart into different gamete are called repulsion or trans configuration.

Kinds of Linkage

- T.H. Morgan found two types of linkage. They are complete linkage and incomplete linkage depending upon the absence or presence of new combination of linked genes.

Complete Linkage

- If the chances of separation of two linked genes are not possible those genes always remain together as a result, only parental combinations are observed. The linked genes are located very close together on the same chromosome such genes do not exhibit crossing over. This phenomenon is called complete linkage. It is rare but has been reported in male Drosophila (Figure 3.7). C.B Bridges (1919) discovered that crossing over is completely absent in some species of male Drosophila.

Incomplete Linkage

- If two linked genes are sufficiently apart, the chances of their separation are possible. As a result, parental and non-parental combinations are observed. The linked genes exhibit some crossing over. This phenomenon is called incomplete linkage. This was observed in maize. (Figure 3.8) It was reported by Hutchinson.

Genetic Mapping

- Genes are present in a linear order along the chromosome. They are present in a specific location called locus (plural: loci). The diagrammatic representation of position of genes and related distances between the adjacent genes is called genetic mapping. It is directly proportional to the frequency of recombination between them. It is also called as linkage map. The concept of gene mapping was first developed by Morgan's student Alfred H Sturtevant in 1913. It provides clues about where the genes lies on that chromosome.

Map distance

- The unit of distance in a genetic map is called a map unit (m.u). One map unit is equivalent to one percent of crossing over (Figure 4.). One map unit is also called a centimorgan (cM) in honour of T.H. Morgan. 100 centimorgan is equal to one Morgan (M). For example: A distance between A and B genes is estimated to be 3.5 map units. It is equal to 3.5 centimorgans or 3.5 % or 0.035 recombination frequency between the genes.

- Genetic maps can be constructed from a series of test crosses for pairs of genes called two point crosses. But this is not efficient because double cross over is missed.

Three point test cross

- A more efficient mapping technique is to construct based on the results of three-point test cross. It refers to analyzing the inheritance patterns of three alleles by test crossing a triple recessive heterozygote with a triple recessive homozygote. It enables to determine the distance between the three alleles and the order in which they are located on the chromosome. Double cross overs can be detected which will provide more accurate map distances.

- Three-point test cross can be best understood by considering following an example.

Uses of genetic mapping

- It is used to determine gene order, identify the locus of a gene and calculate the distances between genes.

- They are useful in predicting results of dihybrid and trihybrid crosses.

- It allows the geneticists to understand the overall genetic complexity of particular organism.

Multiple alleles

- A given phenotypic trait of an individual depends on a single pair of genes, each of which occupies a specific position called the locus on homologous chromosome. When any of the three or more allelic forms of a gene occupy

the same locus in a given pair of homologous chromosomes, they are said to be called multiple alleles.

Characteristics of multiple alleles

- Multiple alleles of a series always occupythe same locus in the homologouschromosome. Therefore, no crossing overoccurs within the alleles of a series.
- Multiple alleles are always responsible forthe same character.
- The wild type alleles of a series exhibitdominant character whereas mutanttype will influence dominance or anintermediate phenotypic effect.
- When any two of the mutant multiplealleles are crossed the phenotype is alwaysmutant type and not the wild type.

Self-sterility in Nicotiana

- In plants, multiple alleles have been reported in association with self-sterility or self-incompatibility. Self-sterility means that thepollen from a plant is unable to germinate on its own stigma and will not be able to bring about fertilization in the ovules of the same plant. East (1925) observed multiple alleles in Nicotiana which are responsible for self-incompatibility or self-sterility. The gene for self-incompatibility can be designated as S, which has allelic series S1, S2, S3, S4 and S5.
- The cross-fertilizing tobacco plants were not always homozygous as S1S1 or S2S2, but all plants were heterozygous as S1S2, S3S4, S5S6. When crosses were made between different S1S2 plants, the pollen tube did not develop normally. But effective pollen tube development was observed when crossing was made with other than S1S2 for example S3S4.

Sex determination in plants

- About 94% of all flowering plants have only one type of individual, which produces flowers with male organs (the stamens) and female organs (the carpels). Such plants are termed as sexually monomorphic. Some 6% of flowering plants which have two separate sexes are called dimorphic. Male

plants produce flowers with stamens and female plants produce flowers with carpels only. Researchers are interested to study the mechanism of sex determination in plants.C.E. Allen (1917) discovered sex determination in plants. Sex determination is a complex process determined by genes, the environment and hormones.

- Sex determination in Silene latifolia (Melandrium album) is of controlled by three distinct regions in a sex chromosome.

    1. Y chromosome determines maleness
    2. X specifies femaleness
    3. X and Y show different segments (I II III IV and V)

Sex determination in papaya

- Recently researchers in Hawaii discovered sex chromosomes in Papaya (Carica papaya, 2n=36). Papaya has 17 pairs of autosomes and one pair of sex chromosomes. Male papaya plants have XY and female plants have XX. Unlike human sex chromosomes, papaya sex chromosomes look like autosomes and it is evolved from autosome. The sex chromosomes are functionally distinct because the Y chromosome carries the genes for male organ development and X bears the female organ developmental genes.

- In papaya sex determination is controlled by three alleles. They are m, M1 and M2 of a single gene.

Genotype	Dominant/ recessive	Modification	Sex
mm	Homozygous recessive	Restrict maleness	Female
M1m	Heterozygous	Induces maleness	Male
M2m	Heterozygous	Induces both the sex	Bisexual (rare)
M1M1 or M2M2 or M1M2	Homozygous/ Heterozygous dominant	Inviable plants	Sterile

Sex Determination in Sphaerocarpos

- Sex determination was first described in the bryophyte Sphaerocarposdonnellii which has heteromorphic chromosomes. The gametophyte is haploid and heteromorphic. The male gametophyte as well as the female gametophyte is an haploid organism with 8 chromosome (n=8). The diploid sporophyte is always heterogametic. Seven autosomes are similar in both male and female gametophyte. But the eighth chromosome of female is X which is larger than the seven autosomes. The eighth chromosome of male is Y which is comparatively smaller than autosomes. The sporophyte containing XY combination produces two types of meiospores, that is some with X and others with Y chromosomes. The meiospores with X chromosomes produce female gametophyte and those with Y chromosome produces male gametophyte.

Sex determination in maize

- Zea mays (maize) is an example for monoecious, which means male and female flowers are present on the same plant. There are two types of inflorescence. The terminal inflorescence which bears staminate florets develops from shoot apical meristem called tassel. The lateral inflorescence which develop pistillate florets from axillary bud is called ear or cob. Unisexuality in maize occurs through the selective abortion of stamens in ear florets and pistils in tassel florets. A substitution of two single gene pairs 'ba' for barren plant and 'ts' for tassel seed makes the difference between monoecious and dioecious (rare) maize plants. The allele for barren plant (ba) when homozygous makes the stalk staminate by eliminating silk and ears. The allele for tassel seed (ts) transforms tassel into a pistillate structure that produce no pollen. The table-3.7 is the resultant sex expression based on the combination of these alleles. Most of these mutations are shown to be defects in gibberellin biosynthesis. Gibberellins play an important role in the suppression of stamens in florets on the ears.

Mutation

- Genetic variation among individuals provides the raw material for the ultimate source of evolutionary changes. Mutation and recombination are the two major processes responsible for genetic variation. A sudden change in the genetic material of an organisms is called mutation. The term mutation was introduced by Hugo de Vries (1901) while he has studying on

the plant, evening primrose (Oenothera lamarkiana) and proposed 'Mutation theory'. There are two broad types of changes in genetic material. They are point mutation and chromosomal mutations.

- Mutational events that take place within individual genes are called gene mutations or point mutation, whereas the changes occur in structure and number of chromosomes is called chromosomal mutation. Agents which are responsible for mutation are called mutagens,that increase the rate of mutation. Mutations can occur either spontaneously or induced. The production of mutants through exposure of mutagens is called mutagenesis, and the organism is said to be mutagenized.

Types of mutation

Let us see the two general classes of gene mutation:

- Mutations affecting single base or basepair of DNA are called point mutation
- Mutations altering the number of copiesof a small repeated nucleotide sequence within a gene

Point mutation

- It refers to alterations of single base pairs of DNA or of a small number of adjacent base pairs

Types of point mutations

- Point mutation in DNA are categorised into two main types. They are base pair substitutions and base pair insertions or deletions. Base substitutions are mutations in which there is a change in the DNA such that one base pair is replaced by another. It can be divided into two subtypes: transitions and transversions. Addition or deletion mutations are actually additions or deletions of nucleotide pairs and also called base pair addition or deletions. Collectively, they are termed indel mutations (for insertion-deletion).
- Substitution mutations or indel mutations affect translation. Based on these different types of mutations are given below.

- The mutation that changes one codon for an amino acid into another codon for that same amino acid are called Synonymous or silent mutations. The mutation where the codon for one amino acid is changed into a codon for another amino acid is called Missense or non-synonymous mutations. The mutations where codon for one amino acid is changed into a termination or stop codon is called Nonsense mutation. Mutations that result in the addition or deletion of a single base pair of DNA that changes the reading frame for the translation process as a result of which there is complete loss of normal protein structure and function are called Frameshift mutations.

Mutagenic agents

- The factors which cause genetic mutation are called mutagenic agents or mutagens. Mutagens are of two types, physical mutagen and chemical mutagen. Muller (1927) was the first to find out physical mutagen in Drosophila.

Physical mutagens:

- Scientists are using temperature and radiations such as X rays, gamma rays, alfa rays, beta rays, neutron, cosmic rays, radioactive isotopes, ultraviolet rays as physical mutagen to produce mutation in various plants and animals.

Temperature:

- Increase in temperature increases the rate of mutation. While rise in temperature, breaks the hydrogen bonds between two DNA nucleotides which affects the process of replication and transcription.

Radiation:

- The electromagnetic spectrum contains shorter and longer wave length rays than the visible spectrum. These are classified into ionizing and non-ionizing radiation. Ionizing radiation are short wave length and carry enough higher energy to ionize electrons from atom. X rays, gamma rays, alfa rays, beta rays and cosmic rays which breaks the chromosomes (chromosomal mutation) and chromatids in irradiated cells. Non-ionizing radiation, UV rays have longer wavelengths and carry lower energy, so they have lower penetrating power than the ionizing radiations. It is used

to treat unicellular microorganisms, spores, pollen grains which possess nuclei located near surface membrane.

Sharbati Sonora

- Sharbati Sonora is a mutant variety of wheat, which is developed from Mexican variety (Sonora 64) by irradiating of gamma rays. It is the work of Dr. M.S.Swaminathan who is known as 'Father of Indian green revolution' and his team.

Castor Aruna

- Castor Aruna is mutant variety of castor which is developed by treatment of seeds with thermal neutrons in order to induce very early maturity (120 days instead of 270 days as original variety).

Chemical mutagens:

- Chemicals which induce mutation are called chemical mutagens. Some chemical mutagens are mustard gas, nitrous acid, ethyl and methyl methane sulphonate (EMS and MMS), ethyl urethane, magnous salt, formaldehyde, eosin and enthrosine. Example: Nitrous oxide alters the nitrogen bases of DNA and disturb the replication and transcription that leads to the formation of incomplete and defective polypeptide during translation.

Comutagens

- The compounds which are not having own mutagenic properties but can enhance the effects of known mutagens are called comutagens.
  Example: Ascorbic acid increase the damage caused by hydrogen peroxide.
  Caffeine increase the toxicity of methotrexate

Chromosomal mutations

- The genome can also be modified on a larger scale by altering the chromosome structure or by changing the number of chromosomes in a cell. These large-scale variations are termed as chromosomal mutations or chromosomal aberrations. Gene mutations are changes that take place

within a gene, whereas chromosomal mutations are changes to a chromosome region consisting of many genes. It can be detected by microscopic examination, genetic analysis, or both. In contrast, gene mutations are never detectable microscopically. Chromosomal mutations are divided into two groups: changes in chromosome number and changes in chromosome structure.

Changes in chromosome number

- Each cell of living organisms possesses fixed number of chromosomes. It varies in different species. Even though some species of plants and animals are having identical number of chromosomes, they will not be similar in character. Hence the number of chromosomes will not differentiate the character of species from one another but the nature of hereditary material (gene) in chromosome that determines the character of species.

- Sometimes the chromosome number of somatic cells are changed due to addition or elimination of individual chromosome or basic set of chromosomes. This condition in known as numerical chromosomal aberration or ploidy. There are two types of ploidy.

I. Ploidy involving individual chromosomes within a diploid set (Aneuploidy)
II. (Ploidy involving entire sets of chromosomes (Euploidy)

Aneuploidy

- It is a condition in which diploid number is altered either by addition or deletion of one or more chromosomes. Organisms showing aneuploidy are known as aneuploids or heteroploids. They are of two types, Hyperploidy and Hypoploidy.

Hyperploidy

- Addition of one or more chromosomes to diploid sets are called hyperploidy. Diploid set of chromosomes represented as Disomy. Hyperploidy can be divided into three types. They are as follows,

Trisomy

- Addition of single chromosome to diploid setis called Simple trisomy(2n+1). Trisomics werefirst reported by Blackeslee (1910) in Daturastramonium (Jimson weed). But later it wasreported in Nicotiana, Pisum and Oenothera.Sometimes addition of two individualchromosome from different chromosomalpairs to normal diploid sets are called Doubletrisomy (2n+1+1).

Tetrasomy

- Addition of a pair or two individual pairs ofchromosomes to diploid set is called tetrasomy(2n+2) and Double tetrasomy (2n+2+2)respectively. All possible tetrasomics areavailable in Wheat.

Pentasomy

- Addition of three individual chromosome from different chromosomal pairs to normal diploid set are called pentasomy (2n+3).

2.Hypoploidy

- Loss of one or more chromosome from the diploid set in the cell is called hypoploidy. It can be divided into two types. They are

Monosomy

- Loss of a single chromosome from the diploid set are called monosomy(2n-1). However loss of two individual or three individual chromosomes are called double monosomy (2n-1-1) and triple monosomy (2n-1-1-1) respectively. Double monosomics are observed in maize.

Nullisomy

- Loss of a pair of homologous chromosomes or two pairs of homologous chromosomes from the diploid set are called Nullisomy (2n-2) and double Nullisomy (2n-2-2) respectively. Selfing of monosomic plants produce nullisomics. They are usually lethal.

Euploidy

- Euploidy is a condition where the organisms possess one or more basic sets of chromosomes. Euploidy is classified as monoploidy, diploidy and polyploidy. The condition where an organism or somatic cell has two sets of chromosomes are called diploid (2n). Half the number of somatic chromosomes is referred as gametic chromosome number called haploid(n). It should be noted that haploidy (n) is different from a monoploidy (x). For example, the common wheat plant is a polyploidy (hexaploidy) 2n=6x=72 chromosomes. Its haploid number (n) is 36, but its monoploidy (x) is 12. Therefore, the haploid and diploid condition came regularly one after another and the same number of chromosomes is maintained from generation to generation, but monoploidy condition occurs when an organism is under polyploidy condition. In a true diploid both the monoploid and haploid chromosome number are same. Thus a monoploid can be a haploid but all haploids cannot be a monoploid.

Polyploidy

- Polyploidy is the condition where an organism possesses more than two basic sets of chromosomes. When there are three, four, five or six basic sets of chromosomes, they are called triploidy (3x) tetraploidy (4x), pentaploidy (5x) and hexaploidy (6x) respectively. Generally, polyploidy is very common in plants but rarer in animals. An increase in the number of chromosome sets has been an important factor in the origin of new plant species. But higher ploidy level leads to death. Polyploidy is of two types. They are autopolyploidy and allopolyploidy

Autopolyploidy

- The organism which possesses more than twohaploid sets of chromosomes derived fromwithin the same species is called autopolyploid.They are divided into two types. Autotriploidsandautotetraploids.

- Autotriploids have three set of its own genomes. They can be produced artificially by crossing between autotetraploid and diploid species. They are highly sterile due to defective gamete formation. Example: The cultivated banana are usually triploids and are seedless having larger fruits than diploids. Triploid sugar beets have higher sugar content than diploids and are resistant to moulds. Common doob grass (Cyanodondactylon) is a natural autotriploid. Seedless watermelon, apple, sugar beet, tomato, banana are man madeautotriploids.

- Autotetraploids have four copies of its own genome. They may be induced by doubling the chromosomes of a diploid species. Example: rye, grapes, alfalfa, groundnut, potato and coffee.

2. Allopolyploidy

- An organism which possesses two or more basic sets of chromosomes derived from two different species is called allopolyploidy. It can be developed by interspecific crosses and fertility is restored by chromosome doubling with colchicine treatment. Allopolyploids are formed between closely related species only.

Significance of Ploidy

- Many polyploids are more vigorous andmore adaptable than diploids.
- Many ornamental plants are autotetraploidsand have larger flower and longer floweringduration than diploids.
- Autopolyploids usually have increase in freshweight due to more water content.
- Aneuploids are useful to determine thephenotypic effects of loss or gain of differentchromosomes.
- Many angiosperms are allopolyploids andthey play a role in an evolution of plants.

Structural changes in chromosome (Structural chromosomal aberration):

- Structural variations caused by addition or deletion of a part of chromosome leading to rearrangement of genes is called structural chromosomal aberration. It occurs due to ionizing radiation or chemical compounds. On the basis of breaks and reunion in chromosomes, there are four types of aberrations. They are classified under two groups.

Changes in the number of the gene loci

- Deletion or Deficiency
- Duplication or Repeat

Changes in the arrangement of gene loci

- Inversion
- Translocation

Deletion or Deficiency

- Loss of a portion of chromosome is calleddeletion. On the basis of location of breakageon chromosome, it is divided into terminaldeletion and intercalary deletion. It occursdue to chemicals, drugs and radiations. It isobserved in Drosophila and Maize.

There are two types of deletion:

Terminal deletion: Single break in any oneend of the chromosome.

- **Intercalary deletion or interstitial deletion**:It is caused by two breaks and reunion ofterminal parts leaving the middle.

- Both deletions are observable during meiotic pachytene stage and polytene chromosome. The unpaired loop formed in the normal chromosomal part at the time of chromosomal pairing. Such loops are called as deficiency loops and it can be seen in meiotic prophase. Larger deletions may lead to lethal effect.

Duplication or Repeat

- The process of arrangement of the same orderof genes repeated more than once in the same chromosome is known as duplication. Due to duplication some genes are present in more than two copies. It was first reported in Drosophila by Bridges (1919) and other examples are Maize and Pea. It is three types.

Tandem duplication

- The duplicated segment is located immediatelyafter the normal segment of the chromosome inthe same order.

Reverse tandem duplication

- The duplicated segment is located immediatelyafter the normal segment but the gene sequenceorder will be reversed.

Displaced duplication

- The duplicated segment is located in the samechromosome, but away from the normalsegment.

Duplications play a major role in evolution.

Inversion

- A rearrangement of order of genes in achromosome by reversed by an angle 1800. Thisinvolve two chromosomal breaks and reunion.During this process there is neither gain nor loss but the gene sequences is rearranged. Inversionwas first reported in Drosophila by Sturtevant(1926). There are two types of inversion,paracentric and pericentric (Figure 3.26).

I. **Paracentric inversion:** An inversion whichtakes place apart from the centromere

II. **Pericentric inversion:** An inversion that includes the centromere. Inversions lead to evolution of a new species.

Translocation

- The transfer of a segment of chromosome to a non-homologous chromosome is called translocation. Translocation should not be confused with crossing over, in which an exchange of genetic material between homologous chromosome takes place. Translocation occurs as a result of interchange of chromosome segments in non-homologous chromosomes. There are three types

  - Simple translocation
  - Shift translocation
  - Reciprocal translocation

Simple translocation

- A single break is made in only one chromosome. The broken segment gets attached to one end of a non-homologous chromosome. It occurs very rarly in nature.

Shift translocation

- Broken segment of one chromosome gets inserted interstitially in a non-homologous chromosome.

Reciprocal translocations

- It involves mutual exchange of chromosomal segments between two non-homologous chromosomes. It is also called illegitimate crossing over. It is further divided into two types.

- **Homozygous translocation**: Both the chromosomes of two pairs are involved in translocation. Two homologous of each translocated chromosomes are identical.

- **Heterozygous translocation**: Only one of the chromosome from each pair of two homologous are involved in translocation, while the remaining chromosome is normal.

Translocations play a major role in the formation of species.

DNA Metabolism in Plants

- As the repository of genetic information, DNA occupies a unique and central place among biological macromolecules. The structure of DNA is a marvelous device for the storage of genetic information. The term "DNA Metabolism" can be used to describe process by which copies of DNA molecules are made (replication) along with repair and recombination.

- **DNA Replication:** In the double helix the two parental strands of DNA separate and each parental strand synthesizes a new complementary strand. DNA replication is semiconservative, i.e each new DNA molecule conserves one original strand.

DNA Repair: How is genomic stability maintained in all living organisms? How do organisms on earth survive? What is essential for their survival?

- DNA is unique because it is the only macromolecule where the repair system exists, which recognises and removes mutations. DNA is subjected to various types of damaging reactions such as spontaneous or environmental agents or natural endogenous threats. Such damages are corrected by repair enzymes and proteins, immediately after the damage has taken place. DNA repair system plays a major role in maintaining the genomic / genetic integrity of the organism. DNA repair systems protect the integrity of genomes from genotoxic stresses.

Plants are sessile. How do they protect themselves from the exposure of

sunlight throughout the day?
Plants have effective DNA repair mechanism to prevent UV damage from sunlight. They produce an enzyme called photolyase, which can repair the thymine dimers and restore the structure of DNA.

- **Recombination:** In cells the genetic information within and among DNA molecule are re-arranged by a process called genetic recombination. Recombination is the result of crossing over between the pairs of homologous chromosomes during meiosis. In earlier classes you have learnt chromosomal recombination. In molecular level it involves breakage and reunion of polynucleotides.

Eukaryotic DNA replication

- Replication starts at a specific site on a DNA sequence known as the Origin of replication. There are more than one origin of replication in eukaryotes. Saccharomyces cerevisiae (yeast) has approximately 400 origins of replication. DNA replication in eukaryotes starts with the assembly of a prereplication complex (preRC) consisting of 14 different proteins. Part of a preRC is a group of 6 proteins called the origin recognition complex (ORC) which acts as initiator in eukaryotic DNA replication. The origin of replication in yeast is called as ARS sites (Autonomously Replicating Sequences). In yeast, ORC was identified as a protein complex which binds directly to ARS elements.

- Replication fork is the site (point of unwinding) of separation of parental DNA strands where new daughter strands are formed. Multiple replication forks are found in eukaryotes. The enzyme helicases are involved in unwinding of DNA by breaking hydrogen bonds holding the two strands of DNA and replication protein A (RPA) prevents the separated polynucleotide strand from getting reattached.

- Topoisomerase is an enzyme which breaks DNAs covalent bonds and removes positive supercoiling ahead of replication fork. It eliminates the torsional stress caused by unwinding of DNA double helix.

- DNA replication is initiated by an enzyme DNA polymerase α / primase which synthesizes short stretch of RNA primers on both leading strand (continuous DNA strand) and lagging strands (discontinuous DNA strand). Primers are needed because DNA polymerase requires a free 3′ OH to

initiate synthesis. DNA polymerase covalently connects the nucleotides at the growing end of the new DNA strand.

- DNA Pol α (alpha), DNA Pol δ (delta) and DNA Pol ε (Epsilon) are the 3 enzymes involved in nuclear DNA replication.

  DNA Pol α – Synthesizes short primers of RNA
  DNA Pol δ – Main Replicating enzyme of cell nucleus
  DNA Pol ε – Extend the DNA Strands in replication fork

DNA Polymerase β does not play any role in the replication of normal DNA. Function -Removing incorrect bases from damagedDNA. It is involved in Base excision repair

Experimental evidence of DNA replication: Taylors Experiment

- J.Herbert Taylor, Philip Woods and WalterHughes demonstrated the semiconservativereplication of DNA in the root cells of Viciafaba. They labelled DNA with 3H Thymidine,a radioactive precursor of DNA and performedautoradiography. They grew root tips in amedium in the presence of radioactive labeled thymidine, so that the radioactivity wasincorporated into the DNA of these cells. Theoutline of this labelled chromosomes appears inthe form of scattered black dots of silver grainson a photographic film.

- In the chromosome of first generation theradioactivity was found to be distributed toboth the chromatids because in the originalstrand of DNA double helix was labeled with radioactivity and the new strand wasunlabelled.

- In the chromosome of the second generation only one of the two chromatids in eachchromosome was radioactive (labelled).
  The results proved the semiconservative method of DNA replication

Translation

- The genetic information in the DNA code is copied onto mRNA bound in ribosomes for making polypeptides. The mRNA nucleotide sequence is decoded into amino acid sequence of the protein which is catalyzed by the ribosome. This process is called translation.

Process of translation

The following are major steps in translation process

Initiation

- The translation begins with the AUG codon(start codon) of mRNA. Translation occurson the surface of the macromolecular arenacalled the ribosome. It is a non-membranousorganelle. During the process of translationthe two subunits of ribosomes unite (combine)together and hold mRNA between them. Theprotein synthesis begins with the reading ofcodons of mRNA. The tRNA brings amino acidto the ribosome, a molecular machine whichunites amino acids into a chain according to theinformation given by mRNA. rRNA plays thestructural and catalytic role during translation.

- A ribosome has one binding site for mRNA and two for tRNA. The two binding sites of tRNA are

  1. P-Site – The peptidyl – tRNA binding siteis one of the tRNA binding site. At this sitetRNA is held and linked to the growing endof the polypeptide chain.

  2. A-Site – The Aminoacyl – tRNA binding site. This is another tRNA binding site which holds the incoming amino acids called aminoacyl tRNA. The anticodons oftRNA pair with the codons of the mRNA inthese sites.

Elongation of polypeptide chain

- The P and A sites are nearby, so that two tRNAform base pairs with adjacent codon. Thepolypeptide chain is formed by the pairingof codons and anticodons according to thenucleotide sequence of the mRNA.

Translators of the genetic code - tRNA

- The tRNA translates the genetic code from the nucleic acid sequence to the amino acid sequence i.e from gene – Polypeptide. When an amino acid is attached to tRNA it is called aminoacylated or charged. This is an energy requiring process whichuses the ATP for its energy requirement. Protein synthesis takes place as the next aminoacyl tRNA binds to the A-Site.

- The translation begins with the AUG codon (start codon) of mRNA. The tRNA which carries first amino acid methionine attach itself to P-site of ribosome. The ribosome adds new amino acids to the growing polypeptides. The second tRNA molecules has anticodons which carries amino acid alanine pairs with the mRNA codon in the A-site of the ribosome. The aminoacids methionine and alanine are close enough so that a peptide bond is formed between them.

- The bond between the first tRNA and methionine now breaks. The first tRNA leaves the ribosome and the P-site is vacant. The ribosome now moves one codon along the mRNA strand. The second t-RNA molecule now occupies the P-site. The third t-RNA comes and fills the A site (serine).Now a peptide bond is formed betweenalanine and serine. The mRNA then movesthrough the ribosome by three bases. Thisexpels deacylated / uncharged tRNA fromP-site and moves peptidyl tRNA into theP-site and empties the A-site.This movementof tRNA from A-site to P-site is said to betranslocation. The translocation requires thehydrolysis of GTP.

- The ribosome (ribozyme - peptidyl transferase) catalyses the formation of peptide bond by adding amino acid to the growing polypeptide chain.

- The ribosome moves from codon to codon along the mRNA in the 5′ to 3′ direction. Amino acids are added one by one translated into polypeptide as dictated by the mRNA. Translation is an energy intensive process. A cluster of ribosomes are linked together by a molecule of mRNA and forming the site of protein synthesis is called as polysomes or polyribosomes.

Termination of polypeptide synthesis

- Eukaryotes have cytosolic proteins called release factors which recognize the termination codon, UAA, UAG, or UGA when it is in the A site. When

the ribosome reaches a stop codon the protein synthesis comes to an end. So ribosomes are the protein making factories of a cell. When the polypeptide is completed the ribosome releases the polypeptide and detaches from the mRNA molecule. Now the ribosome splits into small and large subunits after the release of mRNA.

Alternative Splicing in plants

- It is very useful in regulating gene expression to overcome the environmental stress in plants.

- Alternative splicing is an important mechanism / process by which multiple mRNA's and multiple proteins products can be generated from a single gene. The different proteins generated are called isoforms. There are various modes of alternative splicing. When multiple introns are present in a gene, they are removed separately or as a unit. In certain cases one or more exons which is present between the introns are also removed.

Significance of alternative splicing

- The proteins transcribed from alternatively spliced mRNA containing different aminoacid sequence lead to the generation of protein diversity and biological functions.

- Multiple protein isoforms are formed.

- It creates multiple mRNA transcriptsfrom a single gene. A process of producingrelated proteins from a single gene therebythe number of gene products are increased.

- It plays an important role in plant functions such as stress response and trait selection.The plant adapts or regulates itself to thechanging environment.

Jumping Genes

- This is the nick name of transposable genetic elements. Transposons are the DNA sequences which can move from one position to another position in a genome. This was first reported in 1948 by American Geneticist Barbara McClintock as "mobile controlling element" in Maize. One of the most

significant scientists of 20th century was Barbara McClintock because she gave a shift in gene organization. McClintock was awarded Nobel prize in 1983 for her work on transposons. Barbara McClintock when studying aleurone of single maize kernels, noted the unstable inheritance of the mosaic pattern of blue, brown and red spots due to the differential production of vacuolar anthocyanins.

- In maize plant genome has AC / Ds transposon (AC = Activator, Ds = Dissociation). The activity of AC element is very distinct in maize plant. The transposition in somatic cells results in the changes in gene expression such as variegated pigmentation in maize kernels. Maize genome has transposable elements which regulated the different colour pattern of kernels.

- McClintock's findings concluded that Ds and AC genes were mobile controlling elements. We now call it as transposable elements, a term coined by maize geneticist, Alexander Brink. McClintock gave the first direct experimental evidence that genomes are not static but are highly plastic entities

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Significance of transposons

- They contribute to many visible mutations and mutation rate in an Organism.

- In evolution, they contribute to genetic diversity.

- In genetic research transposons are valuable tools which are used as mutagens, as cloning tags, vehicles for inserting foreign DNA into model organism.

- **Plant genome** – The word genome is defined as the full complement of DNA (including all the genes and the intergenic regions) present in an organism It specifies the entire biological information of an organism. There are three distinct genomes in eukaryotic cells and they are

    - The nuclear genome
    - The mitochondrial genome and
    - The chloroplast genome present only in plants.

- It is a model plant for the study of genetic and molecular aspects of plant development.
- It belongs to mustard family and it is the first flowering plant, where its entire genome is sequenced.

- The two regions of the nucleolar organiser ribosomal DNA which codes for the ribosomal RNA are present at the extremity of chromosomes 2 and 4

- It is Diploid plant having small genome with 2n = 10 chromosomes. Several generations can be produced in one year. So it facilitates rapid genetic analysis. The genome has low repetitive DNA, over 60% of the nuclear DNA have protein coding functions.

- The plant is small, self fertilizes, annual long-day plant with short-life cycle (only 6 weeks), large numbers of seeds are produced and they are easy to be grown in laboratory. It is easy to induce mutations. It has many genomic resources and the transformation can be done easily.

- In 1982, Arabidopsis has successfully completed its life cycle in Microgravity i.e. space. This shows that Human Space Missions with plant companions may be possible.

UNIT – 4 -Principles and processes of Biotechnology

Advancements in Modern Biotechnology

- The modern biotechnology embraces all the genetic manipulations, protoplasmic fusion techniques and the improvements made in the old biotechnological processes. Some of the major advancements in modern biotechnology are described below.

Genetic Engineering

- Genetic engineering or recombinant DNA technology or gene cloning is a collective term that includes different experimental protocols resulting in the modification and transfer of DNA from one organism to another.

- The definition for conventional recombination was already given in Unit II. Conventional recombination involves exchange or recombination of genes between homologous chromosomes during meiosis. Recombination carried out artificially using modern technology is called recombinant DNA technology (r-DNA technology). It is also known as gene manipulation technique. This technique involves the transfer of DNA coding for a specific gene from one organism into another organism using specific agents like vectors or using instrumentslike electroporation, gene gun, liposome mediated, chemical mediated transfers and microinjection.

Steps involved in Recombinant DNA Technology
The steps involved in recombinant DNA technology are:

- Isolation of a DNA fragment containing a gene of interest that needs to be cloned. This is called an insert.

- Generation of recombinant DNA (rDNA) molecule by insertion of the DNA fragment into a carrier molecule called a vector that can self-replicate within the host cell.

- Selection of the transformed host cells that is carrying the rDNA and allowing them to multiply thereby multiplying the rDNA molecule.

Tools for Genetic Engineering

- Now we know from the foregoing discussion that in order to generate recombinant DNA molecule, certain basic tools are necessary for the process. The basic tools are enzymes, vectors and host organisms. The most important enzymes required for genetic engineering are the restriction enzymes, DNA ligase and alkaline phosphatase.

Restriction Enzymes

- The two enzymes responsible for restricting the growth of bacteriophage in Escherichia coli were isolated in the year 1963. One was the enzyme which added methyl groups to DNA, while the other cut DNA. The later was called restriction endonuclease. A restriction enzyme or restriction endonuclease is an enzyme thatcleaves DNA into fragments at or near specific recognition sites within the molecule known as restrictionsites. Based on their mode of action restriction enzymes are classified into Exonucleases and Endonucleases.

- Exonucleases are enzymes which remove nucleotides one at a time from the end of a DNA molecule. e.g. Bal 31, Exonuclease III.

- Endonucleases are enzymes which break the internal phosphodiester bonds within a DNA molecule. e.g. Hind II, EcoRI, Pvul, BamHI, TaqI.

Vectors

- Another major component of a gene cloning experiment is a vector such as a plasmid. A Vector is a small DNA molecule capable of self-replication and is used as a carrier and transporter of DNA fragment which is inserted into it for cloning experiments. Vector is also called cloning vehicle or cloning DNA. Vectors are of two types:

    - Cloning Vector,
    - **Expression Vector.**

- Cloning vector is used for the cloning of DNA insert inside the suitable host cell. Expression vector is used to express the DNA insert for producing specific protein inside the host.

Properties of Vectors

- Vectors are able to replicate autonomously to produce multiple copies of them along with their DNA insert in the host cell.

- It should be small in size and of low molecular weight, less than 10 Kb (kilo base pair) in size so that entry/transfer into host cell is easy.

- Vector must contain an origin of replication so that it can independetly replicate within the host.

- It should contain a suitable marker such as antibiotic resistance, to permit its detection in transformed host cell.

- Vector should have unique target sites for integration with DNA insert and should have the ability to integrate with DNA insert it carries into the genome of the host cell. Most of the commonly used cloning vectors have more than one restriction site. These are Multiple Cloning Site (MCS) or polylinker. Presence of MCS facilitates the use of restriction enzyme of choice.

- The following are the features that are required to facilitate cloning into a vector.

- **Origin of replication (ori):** This is a sequence from where replication starts and piece of DNA when linked to this sequence can be made to replicate within the host cells.

- **Selectable marker:** In addition to ori the vector requires a selectable marker, which helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants.

- **Cloning sites:** In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes.

Types of vector

Few types of vectors are discussed in detail below:

Plasmid

- Plasmids are extra chromosomal, self replicating ds circular DNA molecules, found in the bacterial cells in addition to the bacterial chromosome. Plasmids contain Genetic information for their own replication.

pBR 322 Plasmid

- pBR 322 plasmid is a reconstructed plasmid and most widely used as cloning vector; it contains 4361 base pairs. In pBR, p denotes plasmid, Band R respectively the names of scientist Boliver and Rodriguez who developed this plasmid. The number 322 is the number of plasmid developed from their laboratory. It contains ampR and tetR two different antibiotic resistance genes and recognition sites for several restriction enzymes. (Hind III, EcoRI, BamH I, Sal I, Pvu II, Pst I, Cla I), ori and antibiotic resistance genes. Rop codes for the proteins involved in the replication of the plasmid.

Ti Plasmid

- Ti plasmid is found in Agrobacterium tumefaciens, a bacteria responsible for inducing tumours in several dicot plants. The plasmid carries transfer (tra) gene which help to transfer T- DNA from one bacterium to other bacterial or plant cell. It has Onco gene for oncogenecity,ori gene for origin for replication and inc gene for incompatibility. T-DNA of Ti-Plasmid is stably integrated with plant DNA. Agrobacterium plasmids have been used for introduction of genes of desirable traits into plants.

Transposon as Vector

- Transposons (Transposable elements or mobile elements) are DNA sequence able to insert itself at a new location in the genome without having any sequence relationship with the target locus and hence transposons are called walking genes or jumping genes. They are used as genetic tools for analysis of gene and protein functions, that produce new phenotype on host cell. The use of transposons is well studied in Arabidopsis thaliana and bacteria such as Escherichia coli.

Walking Genes - Gene walking involves the complete sequencing of large more than 1 kb stretches of DNA.

Expression vectors

- Vectors which are suitable for expressing foreign proteins are called expression vectors. This vector consists of signals necessary for transcription and translation of proteins in the host. This helps the host to produce foreign protein in large amounts. Example: pUC 19.

Competent Host (For Transformation with Recombinant DNA)

- The propagation of the recombinant DNA molecules must occur inside a living system or host. Many types of host cells are available for gene cloning which includes E.coli, yeast, animal or plant cells. The type of host cell depends upon the cloning experiment. E.coli is the most widely used organism as its genetic make-up has been extensively studied, it is easy to handle and grow, can accept a range of vectors and has also been studied for safety. One more important feature of E.coli to be preferred as a host cell is that under optimal growing conditions the cells divide every 20 minutes.

- Since the DNA is a hydrophilic molecule,it cannot pass through cell membranes, In order to force bacteria to take up the plasmid, the bacterial cells must first be made competent to take up DNA. This is done by treating them with a specific concentration of a divalent cation such as calcium. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 420C (heatshock) and then putting them back on ice. This enables bacteria to take up the Recombinant DNA.

- For the expression of eukaryotic proteins, eukaryotic cells are preferred because to produce a functionally active protein it should fold properly and post translational modifications should also occur, which is not possible by prokaryotic cell (E.coli).

Methods of Gene Transfer

- The next step after a recombinant DNA molecule has been generated is to introduce it into a suitable host cell. There are many methods to introduce recombinant vectors and these are dependent on several factors such as the vector type and host cell.

- For achieving genetic transformation in plants, the basic pre-requisite is the construction of a vector which carries the gene of interestflanked by the necessary controlling sequences, i.e., the promoter and terminator, and deliver the genes into the host plant. There are two kinds of gene transfer methods in plants. It includes:

    - Direct or vectorless gene transfer
    - Indirect or vector – mediated gene transfer

Direct or Vectorless Gene Transfer

- In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant without the help of a vector. The following are some of the common methods of direct gene transfer in plants.

- **Chemical mediated gene transfer:** Certain chemicals like polyethylene glycol (PEG) and dextran sulphate induce DNA uptake into plant protoplasts.

- **Microinjection:** The DNA is directly injected into the nucleus using fine tipped glass needle or micro pipette to transform plant cells. The protoplasts are immobilised on a solid support (agarose on a microscopic slide) or held with a holding pipette under suction.

- **Electroporation Methods of Gene Transfer:** A pulse of high voltage is applied to protoplasts, cells or tissues which makes transient pores in the plasma membrane through which uptake of foreign DNA occurs.

- **Liposome mediated method of Gene Transfer:** Liposomes the artificial phospholipid vesicles are useful in gene transfer. The gene or DNA is transferred from liposome into vacuole of plant cells. It is carried out by encapsulated DNA into the vacuole. This technique is advantageous because the liposome protects the introduced DNA from being damaged by the acidic pH and protease enzymes present in the vacuole. Liposome and tonoplast of vacuole fusion resulted in gene transfer. This process is called lipofection.

- **Biolistics:** The foreign DNA is coated onto the surface of minute gold or tungsten particles (1-3 µm) and bombarded onto the target tissue or cells using a particle gun (also called as gene gun/micro projectile

gun/shotgun). Then the bombarded cells or tissues are cultured on selected medium to regenerate plants from the transformed cells.

Indirect or Vector-Mediated Gene Transfer

- Gene transfer is mediated with the help of a plasmid vector is known as indirect or vector mediated gene transfer. Among the various vectors used for plant transformation, the Ti-plasmid from Agrobacterium tumefaciens has been used extensively. This bacterium has a large size plasmid, known as Ti plasmid (Tumor inducing) and a portion of it referred as T-DNA (transfer DNA) is transferred to plant genome in the infected cells and cause plant tumors (crown gall). Since this bacterium has the natural ability to transfer T-DNA region of its plasmid into plant genome, upon infection of cells at the wound site, it is also known as the natural genetic engineer of plants.

- The foreign gene (e.g.Bt gene for insect resistance) and plant selection marker gene, usually an antibiotic gene like npt II which confers resistance to antibiotic kanamycin are cloned in the T DNA region of Ti-plasmid in place of unwanted DNA sequences.

Screening for Recombinants

- After the introduction of r-DNA into a suitable host cell, it is essential to identify those cells which have received the r-DNA molecule. This process is called screening. The vector or foreign DNA present in recombinant cells expresses the characters, while the non-recombinants do not express the characters or traits. For this some of the methods are used and one such method is Blue-White Selection method.

Insertional Inactivation - Blue- White Colony Selection Method

- It is a powerful method used for screening of recombinant plasmid. In this method, a reporter gene lacZ is inserted in the vector. The lacZ encodes the enzyme β-galactosidase and contains several recognition sites for restriction enzyme.

- β-galactosidase breaks a synthetic substrates called X-gal (5-bromo-4-chloro-indolyl-β-D-galacto-pyranoside) into an insoluble blue coloured product. If a foreign gene is inserted into lacZ, this gene will be inactivated.

Therefore, no-blue colour will develop (white) because β-galactosidase is not synthesized due to inactivation of lacZ. Therefore, the host cell containing r-DNA form white coloured colonies on themedium contain X-gal, whereas the other cells containing non-recombinant DNA will develop the blue coloured colonies. On the basis of colony colour, the recombinants can be selected.

Antibiotic resistant markers

- An antibiotic resistance marker is a gene that produces a protein that provides cells with resistance to an antibiotic. Bacteria with transformed DNA can be identified by growing on a medium containing an antibiotic. Recombinants will grow on these medium as they contain genes encoding resistance to antibiotics such as ampicillin, chloro amphenicol, tetracycline or kanamycin, etc., while others may not be able to grow in these media, hence it is considered useful selectable marker.

Replica plating technique

- A technique in which the pattern of colonies growing on a culture plate is copied. A sterile filter plate is pressed against the culture plate and then lifted. Then the filter is pressed against a second sterile culture plate. This results in the new plate being infected with cell in the same relative positions as the colonies in the original plate. Usually, the medium used in the second plate will differ from that used in the first. It may include an antibiotic or without a growth factor. In this way, transformed cells can be selected.

Molecular Techniques - Isolation of Genetic Material and Gel Electrophoresis

- Electrophoresis is a separating technique used to separate different biomolecules with positive and negative charges.

Principle

- By applying electricity (DC) the molecules migrate according to the type of charges they have. The electrical charges on different molecules are variable.

+ ve charged Cations will move towards -ve Cathode
-ve charged Anions will move towards +ve Anode

Agarose GEL Electrophoresis

- It is used mainly for the purification of specific DNA fragments. Agarose is convenient for separating DNA fragments ranging in size from a few hundred to about 20000 base pairs. Polyacrylamide is preferred for the purification of smaller DNA fragments. The gel is complex network of polymeric molecules. DNA molecule is negatively charged molecule - under an electric field DNA molecule migrates through the gel. The electrophoresis is frequently performed with marker DNA fragments of known size which allow accurate size determination of an unknown DNA molecule by interpolation. The advantages of agarose gel electrophoresis are that the DNA bands can be readily detected at high sensitivity. The bands of DNA in the gel are stained with the dye Ethidium Bromide and DNA can be detected as visible fluorescence illuminated in UV light will give orange fluorescence, which can be photographed.

Nucleic Acid Hybridization - Blotting Techniques

- Blotting techniques are widely used analytical tools for the specific identification of desired DNA or RNA fragments from larger number of molecules. Blotting refers to the process of immobilization of sample nucleic acids or solid support (nitrocellulose or nylon membranes.) The blotted nucleic acids are then used as target in the hybridization experiments for their specific detection.

Types of Blotting Techniques

Southern Blotting: The transfer of DNA from agarose gels to nitrocellulose membrane.

Northern Blotting: The transfer of RNA to nitrocellulose membrane.

Western Blotting: Electrophoretic transfer of Proteins to nitrocellulose membrane.

Southern Blotting Techniques - DNA

- The transfer of denatured DNA from Agarose gel to Nitrocellulose Blotting or Filter Paper technique was introduced by Southern in 1975 and this technique is called Southern Blotting Technique.

Bioassay for Target Gene Effect

- Target gene is target DNA, foreign DNA, passenger DNA, exogenous DNA, gene of interest or insert DNA that is to be either cloned or specifically mutated. Gene targeting experiments have been targeting the nuclei and this leads to 'gene knock-out'. For this purpose, two types of targeting vectors are used. They are insertion vectors and replacement or transplacement vectors.

- Insertion vectors are entirely inserted into targeted locus as the vectors are linearized within the homology region. Initially, these vectors are circular but during insertion, become linear. It leads to duplication of sequences adjacent to selectable markers.

- The replacement vector has the homology region and it is co-linear with target. This vector is linearized prior to transfection outside the homology region and then consequently a crossing over occurs to replace the endogenous DNA with the incoming DNA.

Transfection: Introduction of foreign nucleic acids into cells by non-viral methods.

Genome Sequencing and Plant Genome Projects

- The whole complement of gene that determine all characteristic of an organism is called genome. The genome may be nuclear genome, mitochondrial genome or plastid genome. Genome of many plants contain both functional and non-expressive DNA proteins. Genome project refer to a project in which the whole genome of plant is analysed using sequence analysis and sequence homology with other plants. Such genome projects have so far been undertaken in Chlamydomonas(algae), Arabidopsis thaliana, rice and maize plants.

- Genome content of an organism is expressed in terms of number of base pairs or in terms of the content of DNA is expressed in c-value.

- **Genome sequencing:** The location of genes on the entire diploid chromosome of an organism.

Evolutionary pattern assessed using DNA.

- In recent years the evolutionary relationship between different plant taxa is assessed using DNA content as well as the similarities and differences in the DNA sequence (sequence homology). Based on such analysis the taxa and their relationship are indicated in cladogram. Such cladogram will show the genetic distance between two taxa. It is also showed antiquity or modernity of any taxon with respect to one another.

Genome editing and CRISPR - Cas9

- Genome editing or gene editing is a group of technologies that has the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome. Several approaches to genome editing have been developed. A recent one is known as CRISPR-Cas9, which is short form of Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9. The CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods.

- Rice, was among the first plants to be used to demonstrate the feasibility of CRISPR-mediated targeted mutagenesis and gene replacement. The gene editing tool CRISPR can be used to make hybrid rice plants that can clone their seed. Imtiyaz Khand and Venkatesan Sundaresan and colleagues reported in a new study which clearly shows one can re-engineer rice to switch it from a sexual to an asexual mode.

RNA Interference (RNAi)

- All characters of organism are the result of expression of different genes which are regions of nuclear DNA. This expression involves transcription and translation. Transcription refers to the copying of genetic information from one strand of the DNA (called sense strand) by RNA. This RNA, as soon as it formed cannot be straight away sent to the cytoplasm to

undertake the process of translation. It has to be edited and made suitable for translation which brings about protein synthesis. One of the main items removed from the RNA strand are the introns. All these changes before translation normally take place whereby certain regions of DNA are silence. However, there is an (RNAi) pathway. RNA interference is a biological process in which RNA molecules inhibit gene expression or translation. This is done by neutralisingtargetd mRNA molecules.

- A simplified model for the RNAi pathway is based on two steps, each involving ribonuclease enzyme. In the first step, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into a short interfering RNA (siRNA) by the RNase II enzymes called Dicer and Drosha. In the second step, siRNAs are loaded into the effector complex RNA-induced silencing complex (RISC). The siRNA is unwound during RISC assembly and the single-stranded RNA hybridizes with mRNA target. This RNAi is seen in plant feeding nematodes.

Transgenic Plants / Genetically Modified Crops (Gm Crops)
Herbicide Tolerant – Glyphosate

- Weeds are a constant problem in crop fields. Weeds not only compete with crops for sunlight, water, nutrients and space but also a carrier for insects and diseases. If left uncontrolled, weeds can reduce crop yields significantly.

Transgenic plants contain a novel DNA introduced into its genome.

- Glyphosate herbicide produced by Monsanto, USA company under the trade name 'Round up' kills plants by blocking the 5-enopyruvate shikimate-3 phosphate synthase (EPSPS) enzyme, an enzyme involved in the biosynthesis of aromatic amino acids, vitamins and many secondary plant metabolites. There are several ways by which crops can be modified to be glyphosate-tolerant.

- One strategy is to incorporate a soil bacterium gene that produces a glyphosate tolerant form of EPSPS. Another way is to incorporate a different soil bacterium gene that produces a glyphosate degrading enzyme.

Advantages of Herbicide Tolerant Crops

- Weed control improves higher crop yields;
- Reduces spray of herbicide;
- Reduces competition between crop plant and weed;
- Use of low toxicity compounds which do not remain active in the soil; and
- The ability to conserve soil structure and microbes.

Herbicide Tolerant - Basta

- Trade name 'Basta' refers to a non-selective herbicide containing the chemical compound phosphinothricin. Basta herbicide tolerant gene PPT (L-phosphinothricin) was isolated from Medicago sativa plant. It inhibits the enzyme glutamine synthase which is involved in ammonia assimilation. The PPT gene was introduced into tobacco and transgenic tobacco produced was resistant to PPT. Similar enzyme was also isolated from Streptomyces hygroscopicus with bar gene encodes for PAT (Phosphinothricin acetyl transferase) and was introduced into crop plants like potato and sugar-beet and transgenic crops have been developed.

Insect resistance - Bt Crops:
Bt Cotton

- Bt cotton is a genetically modified organism (GMO) or genetically modified pest resistant plant cotton variety, which produces an insecticide activity to bollworm.
- Strains of the bacterium Bacillus thuringiensis produce over 200 different Bt toxins, each harmful to different insects. Most Bt toxins are insecticidal to the larvae of moths and butterflies, beetles, cotton bollworms and gatflies but are harmless to other forms of life.
- The genes are encoded for toxic crystals in the Cry group of endotoxin. When insects attack and eat the cotton plant the Cry toxins are dissolved in the insect's stomach.

- The epithelial membranes of the gut block certain vital nutrients thereby sufficient regulation of potassium ions are lost in the insects and results in the death of epithelial cells in the intestine membrane which leads to the death of the larvae.

Advantages

The advantages of Bt cotton are:

- Yield of cotton is increased due to effective control of bollworms.
- Reduction in insecticide use in the cultivation of Bt cotton
- Potential reduction in the cost of cultivation.

Disadvantages

Bt cotton has some limitations:

- Cost of Bt cotton seed is high.
- Effectiveness up to 120 days after that efficiency is reduced
- Ineffective against sucking pests like jassids, aphids and whitefly.
- Affects pollinating insects and thus yield

i. Bt Brinjal

- The Bt brinjal is another transgenic brinjal created by inserting a crystal protein gene (Cry1Ac) from the soil bacterium Bacillus thuringiensis into the genome of various brinjal cultivars. The insertion of the gene, along with other genetic elements such as promoters, terminators and an antibiotic resistance marker gene into the brinjal plant is accomplished using Agrobacterium-mediated genetic transformation. The Bt brinjal has been developed to give resistance against Lepidopteron insects, in particular the Brinjal Fruit and Shoot Borer (Leucinodesorbonalis).

ii. Dhara Mustard Hybrid (DMH)

- DMH -11 is transgenic mustard developed by a team of scientists Centre for Genetic Manipulation of Crop Plants at Delhi University under Government sponsored project. It is genetically modified variety of Herbicide Tolerant (HT) mustard. It was created by using "barnase/ barstar" technology for genetic modification by adding genes from soil bacterium that makes mustard, a self-pollinating plant. DMH -11 contains three genes viz. Bar gene, Barnase and Barstar sourced from soil

bacterium. The bar gene had made plant resistant to herbicide named Basta.

Virus Resistance

- Many plants are affected by virus attack resulting in series loss in yield and even death. Biotechnological intervention is used to introduce viral resistant genes into the host plant so that they can resist the attack by virus. This is by introducing genes that produce resistant enzymes which can deactivate viral DNA.

FlavrSavr Tomato

- Agrobacterium mediated genetic engineering technique was followed to produce Flavr-Savr tomato, i.e., retaining the natural colour and flavor of tomato.

- Through genetic engineering, the ripening process of the tomato is slowed down and thus prevent it from softening and to increase the shelf life. The tomato was made more resistant to rotting by Agrobacterium mediated gene transfer mechanism of introducing an antisense gene which interferes with the production of the enzyme polygalacturonase, which help in delaying the ripening process of tomato during long storage and transportation.

Golden rice - Biofortification

- Golden rice is a variety of Oryza sativa (rice) produced through genetic engineering of biosynthesized beta-carotene, a precursor of Vitamin-A in the edible parts of rice developed by Ingo Potrykus and his group. The aim is to produce a fortified food to be grown and consumed in areas with a shortage of dietary Vitamin-A, which kills so many children under five year age. Golden rice differs from its parental strain by the addition of three beta-carotene biosynthesis genes namely 'psy' (phytoene synthase) from daffodil plant Narcissus pseudonarcissus and 'crt-1' gene from the soil bacterium Erwinia auredorora and 'lyc' (lycopene cyclase) gene from wild-type rice endosperm.

- The endosperm of normal rice, does not contain beta-carotene. Golden-rice has been genetically altered so that the endosperm now accumulates Beta-

carotene. This has been done using Recombinant DNA technology. Golden rice can control childhood blindness - Xerophthalmia.

GM Food - Benefits

- High yield without pest
- 70% reduction of pesticide usage
- Reduce soil pollution problem
- Conserve microbial population in soil

Risks - believed to

- Affect liver, kidney function and cancer
- Hormonal imbalance and physical disorder
- Anaphylactic shock (sudden hypersensitive reaction) and allergies.
- Adverse effect in immune system because of bacterial protein.
- Loss of viability of seeds show in terminator seed technology of GM crops.

Polyhydroxybutyrate (PHB)

- Synthetic polymers are non-degradable and pollute the soil and when burnt add dioxin in the environment which cause cancer. So, efforts were taken to provide an alternative eco-friendly biopolymers. Polyhydroxyalkanoates (PHAs) and polyhydroxybutyrate (PHB) are group of degradable biopolymers which have several medical applications such as drug delivery, scaffold and heart valves. PHAs are biological macromolecules and thermoplastics which are biodegradable and biocompatible.

- Several microorganisms have been utilized to produce different types of PHAs including Gram-positive like Bacillus megaterium, Bacillussubtilis and Corynebacterium glutamicum, Gram-negative bacteria like group of Pseudomonas sp. and Alcaligenes eutrophus.

Polylactic acid (PLA)

- Polylactic acid or polylactide (PLA) is a biodegradable and bioactive thermoplastic. It is an aliphatic polyester derived from renewable resources, such as corn starch, cassava root, chips or starch or sugarcane. For the production of PLA, two main monomers are used: lactic acid, and the cyclic

diester, lactide. The most common route is the ring-opening polymerization of lactide with metal catalysts like tin octoate in solution. The metal-catalyzed reaction results in equal amount of d and polylactic acid.

Green Fluorescent Protein (GFP)

- The green fluorescent protein (GFP) is a protein containing 238 amino acid residues of 26.9 kDa that exhibits bright green fluorescence when exposed to blue to ultraviolet range (395 nm). GFP refers to the protein first isolated from the jellyfish Aequorea victoria. GFP is an excellent tool in biology due to its ability to form internal chromophore without requiring any accessory cofactors, gene products, enzymes or substrates other than molecular oxygen. In cell and molecular biology, the GFP gene is frequently used as a reporter of expression. It has been used in modified forms to make biosensors.

Biopharming

- Biopharming also known as molecular pharming is the production and use of transgenic plants genetically engineered toproduce pharmaceutical substances for use of human beings. This is also called "molecular farming or pharming". These plants are different from medicinal plants which are naturally available. The use of plant systems as bioreactors is gaining more significance in modern biotechnology. Many pharmaceutical substances can be produced using transgenic plants. Example: Golden rice

Bioremediation

- It is defined as the use of microorganisms or plants to clean up environmental pollution. It is an approach used to treat wastes including wastewater, industrial waste and solid waste. Bioremediation process is applied to the removal of oil, petrochemical residues, pesticides or heavy metals from soil or ground water. In many cases, bioremediation is less expensive and more sustainable than other physical and chemical methods of remediation.Bioremediation process is a cheaper and eco-friendly approach and can deal with lower concentrations of contaminants more effectively. The strategies for bioremediation in soil and water can be as follows:

- Use of indigenous microbial population as indicator species for bioremediation process.

- Bioremediation with the addition of adapted or designed microbial inoculants.

- Use of plants for bioremediation - green technology.

Some examples of bioremediation technologies are:

- **Phytoremediation** - use of plants to bring about remediation of environmental pollutants.

- **Mycoremediation** - use of fungi to bring about remediation of environmental pollutants.

- **Bioventing** is the process that increases the oxygen or air flow to accelerate the degradation of environmental pollutants.

- **Bioleaching** is the use of microorganisms in solution to recover metal pollutants from contaminated sites.

- **Bioaugmentation** is the addition of selected microbes to speed up degradation process.

- **Composting** is the process by which the solid waste is composted by the use of microbes into manure which acts as a nutrient for plant growth.

- **Rhizofiltration** is the uptake of metals or degradation of organic compounds by rhizosphere microorganisms.

- **Rhizostimulation** is the stimulation of plant growth by the rhizosphere by providing better growth condition or reduction in toxic materials.

Limitations

- Only biodegradable contaminants can be transformed using bioremediation processes.

- Bioremediation processes must be specifically made in accordance to the conditions at the contaminated site.

- Small-scale tests on a pilot scale must be performed before carrying out the procedure at the contaminated site.

- The use of genetic engineering technology to create genetically modified microorganism or a consortium of microbes for bioremediation process has great potential.

Biofuel: Algal Biofuel

- Algal fuel, also known as algal biofuel, or algal oil is an alternative to liquid fossil fuels, the petroleum products. This use algae as a source of energy-rich oils. Also, algal fuels are an alternative to commonly known biofuel sources obtained from corn and sugarcane. The energy crisis and the world food crisis have initiated interest in algal culture (farming algae) for making biodiesel and other biofuels using land unsuitable for agriculture. Botryococcusbraunii is normally used to produce algal biofuel.

Biological hydrogen production by algae

- The biological hydrogen production with algae is a method of photo biological water splitting. In normal photosynthesis the alga, Chlamydomonas reinhardtii releases oxygen. When it is deprived of sulfur, it switches to the production of hydrogen during photosynthesis and the electrons are transported to ferredoxins. [Fe]-hydrogenase enzymes combine them into the production of hydrogen gas.

Bioprospecting

- Bioprospecting is the process of discovery and commercialization of new products obtained from biological resources. Bioprospecting may involve biopiracy, in which indigenous knowledge of nature, originating with indigenous people, is used by others for profit, without authorization or compensation to the indigenous people themselves.

Biopiracy

- Biopiracy can be defined as the manipulation of intellectual property rights laws by corporations to gain exclusive control over national genetic resources, without giving adequate recognition or remuneration to the original possessors of those resources. Examples of biopiracy include recent patents granted by the U.S. Patent and Trademarks Office to American companies on turmeric, 'neem' and, most notably, 'basmati' rice. All three products are indigenous to the Indo-Pak subcontinent.

Biopiracy of Neem

- The people of India used neem and its oil in many ways to controlling fungal and bacterial skin infections. Indian's have shared the knowledge of the properties of the neem with the entire world. Pirating this knowledge, the United States Department of Agriculture (USDA) and an American MNC (Multi Nation Corporation) W.R.Grace in the early 90's sought a patent from the European Patent Office (EPO) on the "method for controlling of diseases on plants by the aid of extracted hydrophobic neem oil". The patenting of the fungicidal and antibacterial properties of Neem was an example of biopiracy but the traditional knowledge of the Indians was protected in the end.

Biopiracy of Turmeric

- The United States Patent and Trademark Office, in the year 1995 granted patent to the method of use of turmeric as an antiseptic agent. Turmeric has been used by the Indians as a home remedy for the quick healing of the wounds and also for purpose of healing rashes. The journal article published by the Indian Medical Association, in the year 1953 wherein this remedy was mentioned. Therefore, in this way it was proved that the use of turmeric as an antiseptic is not new to the world and is not a new invention, but formed a part of the traditional knowledge of the Indians. The objection in this case US patent and trademark office was upheld and traditional knowledge of the Indians was protected. It is another example of Biopiracy.

Biopiracy of Basmati

- On September 2, 1997, the U.S. Patent and Trademarks Office granted Patent on "basmati rice lines and grains" to the Texas-based company

RiceTec. This broad patent gives the company several rights, including exclusive use of the term 'basmati', as well proprietary rights on the seeds and grains from any crosses. The patent also covers the process of breeding RiceTec's novel rice lines and the method to determine the cooking properties and starch content of the rice grains.

- India had periled the United States to take the matter to the WTO as an infringement of the TRIPS agreement, which could have resulted in major embarrassment for the US. Hence voluntarily and due to few decisions take by the US patent office, Rice Tec had no choice but to lose most of the claims and most importantly the right to call the rice "Basmati". In the year 2002, the final decision was taken. Rice Tec dropped down 15 claims, resulting in clearing the path of Indian Basmati rice exports to the foreign countries. The Patent Office ordered the patent name to be changed to 'Rice lines 867'.

Applications of Biotechnology

- Biotechnology is one of the most important applied interdisciplinary sciences of the 21st century. It is the trusted area that enables us to find the beneficial way of life.

- Biotechnology has wide applications in various sectors like agriculture, medicine, environment and commercial industries.

- This science has an invaluable outcome like transgenic varieties of plants e.g. transgenic cotton (Bt-cotton), rice, tomato, tobacco, cauliflower, potato and banana.

- The development of transgenics as pesticide resistant, stress resistant and disease resistant varieties of agricultural crops is the immense outcome of biotechnology.

- The synthesis of human insulin and blood protein in E.coli and utilized forinsulin deficiency disorder in human is a breakthrough in biotech industries in medicine.

- The synthesis of vaccines, enzymes, antibiotics, dairy products and beverages are the products of biotech industries.

- Biochip based biological computer is one of the successes of biotechnology.

- Genetic engineering involves genetic manipulation, tissue culture involves aseptic cultivation of totipotent plant cell into plant clones under controlled atmospheric conditions.

- Single cell protein from Spirulina is utilized in food industries.

- Production of secondary metabolites, biofertilizers, biopesticides and enzymes.

- Biomass energy, biofuel, Bioremediation, phytoremediation for environmental biotechnology.

UNIT – 5- Plant Tissue Culture

- Growing plant protoplasts, cells, tissues or organs away from their natural or normal environment, under artificial condition, is known as Tissue Culture. It is also known as in vitro (In vitro is a Latin word, it means that - in glass or in test-tube) growth of plant protoplasts, cells, tissues and organs. A single explant can be multiplied into several thousand plants in short time period and space under controlled conditions. Tissue culture techniques are oft en used for commercial production of plants as well as for plant research. Plant tissue culture serves as an indispensable tool for regeneration of transgenic plants. Apart from this some of the main applications of Plant tissue culture are clonal propagation of elite varieties, conservation of endangered plants, production of virus-free plants, germplasm preservation, industrial production of secondary metabolites. etc., In this chapter let us discuss the history, techniques, types , applications of plant tissue culture and get aware on ethical issues. Gottlieb Haberlandt (1902) the German Botanist proposed the concept Totipotency and he was also the first person to culture plant cells in artificial conditions using the mesophyll cells of Lamium purpureum in culture medium and obtained cell proliferation. He is regarded as the father of tissue culture.

Basic concepts of Tissue Culture

- Basic concepts of plant tissue culture are totipotency, differentiation, dedifferentiation and redifferentiation.

Totipotency

- The property of live plant cells that they have the genetic potential when cultured in nutrient medium to give rise to a complete individual plant.

Differentiation

- The process of biochemical and structural changes by which cells become specialized in form and function.

Redifferentiation

- The further differentiation of already differentiated cell into another type of cell. For example, when the component cells of callus have the ability to

form a whole plant in a nutrient medium, the phenomenon is called redifferentiation.

Dedifferentiation

- The phenomenon of the reversion of mature cells to the meristematic state leading to the formation of callus is called dedifferentiation. These two phenomena of redifferentiation and dedifferentiation are the inherent capacities of living plant cells or tissue. This is described as totipotency.

Plant Tissue Culture (PTC):

- Plant tissue culture is used to describe the in vitro and aseptic growth of any plant part on a tissue culture medium. This technology is based on three fundamental principles:

- The plant part or explant must be selectedand isolated from the rest of plant body.

- The explant must be maintained in controlled physically (environmental) and chemicallydefined (nutrient medium) conditions.

Explant: The tissue taken from a selected plant transferred to a culture medium often to establish a new plant.

Laboratory Facilities for PTC

For PTC, the laboratory must have the following facilities:

- Washing facility for glassware and ovens fordrying glassware.

- Medium preparation room with autoclave,electronic balance and pH meter.

- Transfer area sterile room with laminarair-flow bench and a positive pressureventilation unit called High EfficiencyParticulate Air (HEPA) filter to maintainaseptic condition.

- **Culture facility:** Growing the explantinoculated into culture tubes at 22-28° Cwith illumination of light 2400 lux, with aphotoperiod of 8-16 hours and a relativehumidity of about 60%.

Technique Involved in PTC
Sterilization:

- Sterilization is the technique employed to getrid of microbes such as bacteria and fungi in theculture medium, vessels and explants.

- **Maintenance of Aseptic Environment:**During in vitro tissue culture maintenance ofaseptic environmental condition should befollowed, i.e., sterilization of glassware, forceps,scalpels, and all accessories in wet steamsterilization by autoclaving at 15 psi (121°C)for 15 to 30 minutes or dipping in 70% ethanolfollowed by flaming and cooling.

- **Sterilization of culture room:** Floor andwalls are washed first with detergent and thenwith 2% sodium hypochlorite or 95% ethanol.The cabinet of laminar airflow is sterilized byclearing the work surface with 95% ethanol andthen exposure of UV radiation for 15 minutes.

- **Sterilization of Nutrient Media:** Culturemedia are dispensed in glass containers, plugged with non-absorbent cotton or sealed with plastic closures and then sterilized using autoclave at15 psi (121°C) for 15 to 30 minutes. The plantextracts, vitamins, amino acids and hormonesare sterilized by passing through Millipore filterwith 0.2 mm pore diameter and then addedto sterilized culture medium inside LaminarAirflow Chamber under sterile condition.

- **Sterilization of Explants:** The plantmaterials to be used for tissue culture should besurface sterilized by first exposing the materialin running tap water and then treating it insurface sterilization agents like 0.1% mercuricchloride, 70% ethanol under aseptic conditioninside the Laminar Air Flow Chamber.

Media Preparation

- The success of tissue culture lies in thecomposition of the growth medium, plantgrowth regulators and culture conditionssuch as temperature, pH, light and humidity.No single medium is capable of maintainingoptimum growth of all plant tissues. Suitablenutrient medium as per the principle of tissueculture is prepared and used.

- MS nutrient medium (Murashige and Skoog 1962) is commonly used. It has carbon sources, with suitable vitamins and hormones. The media formulations available for plant tissue culture other than MS are B5 medium (Gamborg.et.al 1968), White medium (white 1943), Nitsch's medium (Nitsch & Nitsch 1969). A medium may be solid or semisolid or liquid. For solidification, a gelling agent such as agar is added.

Agar: A complex mucilaginous polysaccharide obtained from marine algae (sea weeds) used as solidifying agent in media preparation

Culture condition
Ph

- The pH of medium is normally adjusted between 5.6 to 6.0 for the best result.

Temperature

- The cultures should be incubated normally at constant temperature of 25°C ± 2°C for optimal growth.

Humidity and Light Intensity

- The cultures require 50-60% relative humidity and 16 hours of photoperiod by the illumination of cool white fluorescent tubes of approximately 1000 lux.

Aeration

- Aeration to the culture can be provided by shaking the flasks or tubes of liquid culture on automatic shaker or aeration of the medium by passing with filter-sterilized air.

Induction of Callus

- Explant of 1-2 cm sterile segment selected from leaf, stem, tuber or root is inoculated (transferring the explants to sterile glass tube containing nutrient medium) in the MS nutrient medium supplemented with auxins and incubated at 25°C ± 2°C in an alternate light and dark period of 12 hours to induce cell division and soon the upper surface of explant develops into

callus. Callus is a mass of unorganized growth of plant cells or tissues in in vitro culture medium

Embryogenesis

- The callus cells undergoes differentiation and produces somatic embryos, known as Embryoids. The embryoids are sub-cultured to produce plantlets.

Hardening

- The plantlets developed in vitro require ahardening period and so are transferred togreenhouse or hardening chamber and then tonormal environmental conditions.

- Hardening is the gradual exposure of in vitrodeveloped plantlets in humid chambers indiffused light for acclimatization so as to enablethem to grow under normal field conditions.

Types of Plant tissue cultures

Based on the explants some other plant tissue culture types are

- Organ culture
- Meristem culture
- Protoplast culture
- Cell culture.

Organ culture

- The culture of embryos, anthers, ovaries, roots, shoots or other organs of plants on culture media.

Meristem Culture:

- The culture of any plant meristematic tissue onculture media.

Protoplast Culture:

- Protoplasts are cells without a cell wall, butbounded by a cell membrane or plasmamembrane. Using protoplasts, it is possible toregenerate whole plants from single cells andalso develop somatic hybrids. The steps involved in protoplast culture.

Isolation of protoplast:

- Small bits of planttissue like leaf tissue are used for isolation ofprotoplast. The leaf tissue is immersed in 0.5%Macrozyme and 2% Onozuka cellulase enzymesdissolved in 13% sorbitol or mannitol at pH 5.4.It is then incubated over-night at 25°C. After agentle teasing of cells, protoplasts are obtained,and these are then transferred to 20% sucrosesolution to retain their viability. They are thencentrifuged to get pure protoplasts as differentfrom debris of cell walls.

Fusion of protoplast:

- It is done through theuse of a suitable fusogen. This is normally PEG(Polyethylene Glycol). The isolated protoplastare incubated in 25 to 30% concentration ofPEG with Ca++ ions and the protoplast showsagglutination (the formation of clumps of cells)and fusion.

Culture of protoplast:

- MS liquid medium is used with some modification in droplet, platingor micro-drop array techniques. Protoplastviability is tested with fluorescein diacetatebefore the culture. The cultures are incubated incontinuous light 1000-2000 lux at 25°C. The cellwall formation occurs within 24-48 hours andthe first division of new cells occurs between2-7 days of culture.

Selection of somatic hybrid cells:

- Thefusion product of protoplasts without nucleus ofdifferent cells is called a cybrid. Following thisnuclear fusion happen. This process is calledsomatic hybridization.

Cell Suspension Culture

- The growing of cells including the cultureof single cells or small aggregates of cellsin vitroin liquid medium is known as cellsuspension culture. The cell suspension isprepared by transferring a portion of callus tothe liquid medium and agitated using rotary shaker instrument. The cells are separated from the callus tissue and used for cell suspension culture.

Production of Secondary Metabolites

- Cell suspension culture can be useful for the production of secondary metabolites like alkaloids, flavonoids, terpenoids, phenolic compounds and

recombinant proteins. Secondary metabolites are chemical compounds that are not required by the plant for normal growth and development but are produced in the plant as 'byproducts' of cell metabolism. For Example: Biosynthesis and isolation of indole alkaloids from Catharanthus roseusplant cell culture.

- The process of production of secondary metabolites can be scaled up and automated using bio-reactors for commercial production. Many strategies such as biotransformation, elicitation and immobilization have been used to make cell suspension cultures more efficient in the production of secondary metabolites. Few examples of industrially important plant secondary metabolites are listed below in the table

Secondary metabolites	Plant source	Uses
Digoxin	Digitalis purpuria	Cardiac tonic
Codeine	Papaver sominiferum	Analgesic
Capsaicin	Capsicum annum	Rheumatic pain treatment
Vincristine	Catharanthus roseus	Anti-carcinogenic
Quinine	Cinchona officinalis	Antimalarial

Somatic Embryogenesis

- Somatic embryogenesis is the formation of embryos from the callus tissue directly and these embryos are called Embryoids or from the in vitro cells directly form pre-embryonic cells which differentiate into embryoids.

Applications

- Somatic embryogenesis provides potentialplantlets which after hardening period can establish into plants.

- Somatic embryoids can be be used for theproduction of synthetic seeds.

- Somatic embryogenesis is now reportedin many plants such as Allium sativum, Hordeum vulgare,Oryza sativa, Zea mays and this possible in any plant.

Synthetic seeds are produced by encapsulation of embryoids in agarose gel or calcium alginate.

Applications of Plant Tissue Culture

Plant tissue culture techniques have several applications such as:

- Improved hybrids production throughsomatic hybridization.
- Somatic embryoids can be encapsulatedinto synthetic seeds (synseeds). Theseencapsulated seeds or synthetic seeds helpin conservation of plant biodiversity.
- Production of disease resistant plantsthrough meristem and shoot tip culture.
- Production of stress resistant plants likeherbicide tolerant, heat tolerant plants.
- Micropropagation technique to obtainlarge numbers of plantlets of both crop andtree species useful in forestry within a shortspan of time and all through the year.
- Production of secondary metabolites fromcell culture utilized in pharmaceutical,cosmetic and food industries.

Somaclonal variations: Somatic variations found in plants regenerated in vitro (i.e. variations found in leaf, stem, root, tuber or propagule)

Gametoclonal variations: Gametophytic variations found in plants regenerated in vitro gametic origin (i.e. variations found in gametes and gametophytes)

Artificial Seed

- Artificial seeds or synthetic seeds (synseeds) are produced by using embryoids (somatic embryos) obtained through in vitro culture. They may even be derived from single cells from any part of the plant that later divide to form cell mass containing dense cytoplasm, large nuclceus, starch grains, proteins, and oils etc., To prepare the artificial seeds different inert materials are used for coating the somatic embryoids like agrose and sodium alginate

Advantages of Artificial seeds

Artificial seeds have many advantages over the true seeds

- Millions of artificial seeds can be producedat any time at low cost.
- They provide an easy method to producegenetically engineered plants with desirable traits.
- It is easy to test the genotype of plants.
- They can potentially stored for long timeunder cryopreservation method.
- Artificial seeds produce identical plants
- The period of dormancy of artificial seeds isgreatly reduced, hence growth is faster witha shortened life cycle.

Virus-free plants

- The field grown plants like perennial crops, usually are infected by variety of pathogens like fungi, bacteria, mycoplasma, viruses which cause considerable economic losses. Chemical methods can be used to control fungal and bacterial pathogens, but not viruses generally.
- Shoot meristem tip culture is the method to produce virus-free plants, because the shoot meristem tip is always free from viruses.

Germplasm Conservation

- Germplasm conservation refers to the conservation of living genetic resources like pollen, seeds or tissue of plant material maintained for the purpose of selective plant breeding, preservation in live condition and used for many research works.
- Germplasm conservation resources is a part of collection of seeds and pollen that are stored in seed or pollen banks, so as to maintain their viability and fertility for any later use such as hybridization and crop improvement. Germplasm conservation may also involve a gene bank, DNA bank of elite breeding lines of plant resources for the maintenance of biological diversity and also for food security.

Cryopreservation (–195.C)

- Cryopreservation, also known asCryo-conservation, is a process by which protoplasts, cells, tissues, organelles, organs, extracellular matrix, enzymes or any other biological materials are subjected to preservation by cooling to very low temperature of –196°C using liquid nitrogen. At this extreme low temperature any enzymatic or chemical activity of the biological material will be totally stopped and this leads to preservation of material in dormant status. Later these materials can be activated by bringing to room temperature slowly for any experimental work.

- Protective agents like dimethyl sulphoxide, glycerol or sucrose are added before cryopreservation process. These protective agents are called cryoprotectants, since they protect the cells, or tissues from the stress of freezing temperature.

Intellectual Property Right (IPR)

- Intellectual property right (IPR) is a category of property that includes intangible creation of the human intellect, and primarily consists of copyrights, patents, and trademarks. It also includes other types of rights, such as trade secrets, publicity rights, moral rights, and rights against unfair competition.

- In biotechnology, the transformedmicroorganisms and plants and technologies for the production of commercial productsare exclusively the property of the discoverer.

- The discoverer has the full rights on hisproperty. It should not be neglected by theothers without legal permission.

- The right of discoverer must be protectedand it does by certain laws framed by acountry.

- The IPR is protected by different wayslike patents, copyrights, trade secrets and trademarks, designs and geographical indications.

Patents

- It is a special right to the discoverer/inventorthat has been granted by the governmentthrough legislation for trading new articles.
- A patent is a personal property whichcan be licensed or sold by the person ororganisation just like any other property.
- Patent terms give the inventor the rights toexclude others from making, using or sellinghis invention.
- It is difficult to keep secret certain inventionsand therefore, guidance should be obtainedfrom a qualified patent attorney.
- A patent consists of three parts: the grant,specifications and claims.
- The grant is filled at the patent office whichis not published. It is a signed document,actually the agreement that grants patentright to the inventor.
- The specification and claims are publishedas a single document which is made publicfrom the patent office. The specification partis narrative in which the subject matter ofinvention is described as how the inventionwas carried out.
- The claim specifically defines the scope ofthe invention to be protected by the patentwhich the others may not practice.

Biosafety and Bioethics

- Advances in biotechnology and their applications are mostly associated with controverisies. This is because the major part of the modern biotechnology deals with genetic manipulations. ELSI which represents ethical, legal and social implications of biotechnology broadly covers the relationship between biotechnology and society with particular reference to ethical and legal aspects.

Biosafety

- Biosafety is the prevention of large-scale loss of biological integrity, focusing both on ecology and human health. These prevention mechanisms

include conduction of regular reviews of the biosafety in laboratory settings, as well as strict guidelines to follow. Biosafety is used to protect from harmful incidents. Many laboratories handling biohazards employ an ongoing risk management assessment and enforcement process for biosafety. Failures to follow such protocols can lead to increased risk of exposure to biohazards or pathogens. Human error and poor techniques contribute to unnecessary exposure to hazards and compromise the best safeguards set into place for protection.

Potential risks and consideration for safety aspects

- Pathogenicity of living organisms andviruses - natural and genetically modified-to infect humans, animals and plants tocause diseases.
- Toxicity of allergy associated with microbialproduction.
- Increasing number of antibiotic resistantpathogenic microorganisms.
- Problems associated with the disposal ofspent microbial biomass and purification ofeffluent from biotechnological process.
- Safety aspects associated with contamination, infection or mutation of process strains.
- Safety aspects associated with the industrialuse of microorganisms containing in vitrorecombinants.

Biosafety guidelines are being implemented by:

- The Institutional Bio-safety Committees(IBSCs) monitor the research activity atinstitutional level.
- The Review Committee on GeneticManipulation (RCGM) functioning inthe Department of Biotechnology (DBT)monitors the risky research activities in thelaboratories.
- The Genetic Engineering ApprovalCommittee (GEAC) of Ministry ofEnvironment and Forest has the powerto permit the use of Genetically ModifiedOrganism (GMO) at commercial level andopen field trials of

transgenic materialsincluding agricultural crops, industrialproducts and health care products.

Bioethics - Ethical, Legal and Social Implications (ELSI)

- Bioethics refers to the study of ethical issues emerging from advances in biology and medicine. It is also a moral discernment as it relates to medical policy and practice. Bioethicists are concerned with the ethical questions that arise in the relationships amonglife sciences, biotechnology and medicine. It includes the study of values relating to primary care and other branches of medicine.The scope of bioethics is directly related to biotechnology, including cloning, gene therapy, life extension, human genetic engineering, astroethics life in space, and manipulation of basic biology through altered DNA, RNA and proteins. These developments in biotechnology will affect future evolution, and may require new principles, such as biotic ethics, that values life and its basic biological characters and structures.

- The Ethical, Legal, and Social Implications (ELSI) program was founded in 1990 as an integral part of the Human Genome Project. The mission of the ELSI program was to identify and address issues raised by genomic research that would affect individuals, families, and society. A percentage of the Human Genome Project budget at the National Institutes of Health and the U.S. Department of Energy was devoted to ELSI research.

Ethical issues in Genomic Research

- Privacy and fairness in the use of geneticinformation, including the potential forgenetic discrimination in employment andinsurance.

- The integration of new genetic technologies,such as genetic testing, into the practice ofclinical medicine.

- Ethical issues surrounding the design andconduct of genetic research with people,including the process of informed consent.

Genetic Engineering Appraisal Committee (GEAC)

- GEAC is an apex body under Ministry of Environment, Forests and Climate change for regulating manufacturing, use, import, export and storage of hazardous microbes or genetically modified organisms (GMOs) and cells in

the country. It was established as an apex body to accord approval of activities involving large scale use of hazardous microorganismsand recombinants in research and industrial production. The GEAC is also responsible for approval of proposals relating to release of genetically engineered organisms and products into the environment including experimental field trials (Biosafety Research Level trial-I and II known as BRL-I and BRL-II).

Future of Biotechnology

- Biotechnology has become a comprehensive scientific venture from the point of academic and commercial angles, within a short time with the sequencing of human genome and genome of some important organisms. The future developments in biotechnology will be exciting. Thus the development in biotechnology will lead to a new scientific revolution that would change the lives and future of people. Like industrial and computer revolution, biotechnological revolution will also promise major changes in many aspects of modern life.
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