MUTATION

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MUTATION

- Mutation was first discovered by Wright (1791) in male lamb which was short leg
- The term mutation was introduced by Hugo de Vries (1900) and he was reported in Oenothera.
- Morgan (1910) was discovered the white eye mutant in Drosophila.
- Mutagenic action of X-rays in Drosophila was discovered by Muller (1927). He was awarded Nobel Prize (1946) for this work.
- The mutagenic action of gamma and X-rays was discovered by Stadler (1928) in barley and maize.
- In 1946, Auerbach and Robson reported that nitrogen mustard produced mutation in Drosophila.
- "Mutation refers to sudden heritable changes in the phenotype of an organism", it is known as mutation.
- 'Mutations occur in natural population without any treatment by man at a low rate, and is known as spontaneous mutation'. There are some example of spontaneous mutation such as (i) waxy locus of maize are highly mutable, (ii) yellow body locus of Drosophila show hight mutation rate.
- The frequency of spontaneous mutations is generally one in 10 lacs (10^{-6}) or between 10^{-7} and 10^{-4}).
- Some genes show high rates of spontaneous mutation, called mutable gene, while some still other genes supress the mutation of other gene of the genome, called antimutator gene.
- Mutator gene may promote spontaneous mutation of other genes (e.g., Dt- dotted) on chromosome 9 in maize increases the mutation frequency of 'a' (colourless aleurone) to 'A' (coloured aleurone) which present in chromosome-3.

CHARACTERISTICS OF MUTATIONS

- Mostly mutant alleles are recessive to their wild type or normal alleles. Some mutation are dominant, e.g., notch wing in Drosophila.
- Mutations are random events in terms of time of their occurrence and the gene in which they occur.
- A mutation occurring in a somatic cell, called somatic mutation, it also occur in axillary bud, called bud mutation.
- Mostly mutation have harmful effects, the beneficial mutation is about 0.1% which are useful in crop improvement.
- Most mutant alleles are pleiotropic.
- The rate of spontaneous mutation of any gene is ranging between $10^{-7}$and 10^{-4}. , which are varies from one gene to another, e.g., gene of waxy locus of maize are highly mutable.
- Such gene which show high rate of spontaneous mutation, called mutable genes, while such genes increase the spontaneous mutation rate of the other genes of an genome, called mutator genes, but some genes suppress the mutation of some other genes of the genome, called antimutator genes.
- The frequency of induced mutations is affected by the environmental conditions during the irradiation with X-rays or gamma-rays, e.g., high mutation rate at low moisture content (4-5% in wheat, barley and oats) than 10-12% normal moisture content.
- The frequency of induced mutations are high in S phase of meiosis division than other stages.
- Some sites within genes show very high rate of mutation, while some other sites show little or no mutation. The high mutable sites within a gene are commonly known as hot spots.
- A mutation from the wild type allele to a mutant allele, called forward mutation, while a mutation from a mutant allele to the normal allele, is called reverse mutation.

CLASSIFICATION OF MUTATIONS

1. The direction of mutation

The direction of mutation are grouped into two classes: (i) A mutation from the wild type allele to a mutant allele is called forward mutation, and (ii) Some mutant alleles do not mutate back, is known as reverse mutation.

2.The cause of mutation

Mutations are classified on the cause of their origin. Spontaneous mutations occur naturally without any known cause, and induce mutations originate by treatment with certain physical and chemical mutagens.

3. Dominance relationship

Mutation may be (i) dominant mutation, (ii) recessive mutation, (iii) occasionally codominant mutation, e.g., blood group antigens, or (iv) incompletely dominant mutation, to their wild type alleles.

4. Tissue of origin

Mutation may be classified on the basis of tissues in which they occur. (i) A mutation occurring in a somatic cell which is not give rise to gametes, called somatic mutation. (ii) A somatic mutation occurs in an axillary bud, called bud mutation. (iii) when mutation occur in reproductive tissues, is called germinal mutation.

5. Effect of survival

Many mutation are classified on the basis of their effect on survival of the organisms. (i) All the individuals carrying such mutation are killed. Dominant lethal can not survive in the heterozygous state. Only recessive lethal have to be considered active in their lethal action. Recessive lethal would killed the individuals that carry them in homozygous state, e.g., albino chlorophyl mutation, it is called lethal mutation. (ii) Such lethal killed more than 50% of the individuals, is known as sub lethal mutation. (iii) It kill less than 50% of the individuals, is called sub vital mutation. (iv) All such mutants are survive, but it occur in a much lower frequency (0.1%), is called vital mutation. (v) Such mutants are enhance the performance of the individuals, is called super vital. Only vital and super vital mutations are of direct use for crop improvement programme.

6. Type of character affected

Mutations may be classifies on the basis of their type of character affected.(i) A mutation which alters a morphological characters in the presence of mutant allele, and visually detected, is called visible mutation. When mutation do not changes in morphological characters, but prevent the production of a biochemical by the organism, is called biochemical mutation.

7. Quantum of morphological effect produced

Mutations may be classified on the basis of quantum of phenotypic change produced by them. (i) Mutation with distinct morphological changes in phenotype are referred to as machromutation. Such mutations are found in qualitative characters. Therefore, it is also called oligogenic mutation. (ii) Mutations with invisible phenotypic changes are known as michromutation. Such mutation are observed quantitative characters. So it is called polygenic mutation.

8. Cytological basis

Mutations are classified on their cytological bases. (i) Mutations may be produced by changes in chromosome structure or even in chromosome number, they are termed as chromosomal mutation. (ii) Mutations produced by changes in the base sequences of genes (as a result of base pair transition or transversion, deletion, duplication or inversion, etc.) are known as gene or point mutation. (ii) Mutations are s Associated with changes in mitochondrial or chloroplast DNA, is called cytoplasmic mutation.

9. Molecular basis

Mutations produced by changes in the base sequences of genes (as a result of base pair transition or transversion, deletion, duplication or inversion, etc.) are known as gene or point mutation. Point mutations can be subdivided into the following classes on the basis of molecular change associated with them. (i) When a single base in a DNA molecule is replaced by another base, is called base substitution mutation. (ii) Replacement of one purine by another purine, e.g., A→G or vice versa. Replacement of one pyrimidine by another pyrimidine, e.g., T→C or vice versa. As a result, only one amino acid is altered in the concerned protein, is called transition mutation. (iii) A pyrimidine (T or C) is replaced by a purine (A or G) and vice versa. As a result, one transversion event insertion of one or affects only one codon and hence replaced only one amino acid in the concerned protein. is known as transversion mutation. (iv) One or more bases are deleted or lost from a gene, called deletion mutation. (v) Insertion of one or more bases in a DNA molecules is called base addition mutation. (vi) Frame shift mutation change all the amino acids of the concerned protein which are located beyond the point of addition/deletion of the bases. Generally, proteins produced by frame shift mutation are non-functional. Hence, such mutation are much more deleterious than those produce by base substitution.

MUTAGENS

Agents which are use to induce mutation, are known as mutagens.

PHYSICAL MUTAGENS

1. **Ionizing radiations**
   - **(a) Particulate radiation:** α-rays (DI), β-rays (SI), Fast neutrons (DI), Thermal neutrons (DI).
   - **(b) Non particulate radiation (Electromagnetic radiation):** X-rays (SI), and γ-rays (SI)
2. **Non-ionizing radiation:** UV radiation

CHEMICAL MUTAGENS

1. **Alkylating agents:** Mustard gas or Sulphur mustard, Nitrogen mustard, Ethylmethene Sulphonate (EMS), Methylmethene sulphonate (MMS), Ethylethene sulphonate (EES),N-Methyl-N'-nitro-N'-nitrosogunanidine (NTG).
2. **Base Analogues:** 5-Bromouracil (5-BU), 2-Aminopurine (2-AP).
3. **Acridine Dyes:** Acriflavin, Proflavin, Acridine orange, Ethidium bromide.
4. **Deamination agents:** Nitrous acid (NHO2)
5. **Other chemical mutagens:** Hydroxylamine (HA), Sodium azide, DNA sequences.

PHYSICAL MUTAGENS:

- इसका मुख्य स्रोत परमाणु (atom) होता है। एक atom में एक nucleus (Neutron and positively charge protons) होता है। nucleus के arbits में negatively charged electron (e-) होते है।
- Atoms में bounded energy होती है। एक unstable atom अपने को स्थिरता बनाये रखने के लिए energy या particles को उत्सर्जित करता है, जिसे nuclear decay (ह्रास) कहते है।
- इस nuclear ह्रास से उत्पन्न energetic atomic particles या electromagnetic wave को ही radiation कहते है। जब इसीradiation द्वारा पौधो या जीवो को treatment किया जाता है, तो उसे irradiation कहते है।
- जब High energy short waves वाला radiation किसी पदार्थ के अन्दर electric या magnetic disturbances उत्पन्न करता है, तो उसे electromagnetic radiation कहते है।

Ionizing radiation:

- जब किसी परमाणु से एक इलेक्ट्रान की कमी हो जाती है, तो उसमें धनात्मक आवेश उत्पन्न होता है। जबकि एक इजेक्ट्रान की बढोत्तरी होती है, तो उसमें ऋणात्मक आवेश उत्पन्न होता है। इसे ही ionization कहते है।
- Radiation अपने मार्ग मे पड़ने वालेatoms मे ionization और excitation दोनो उत्पन्न करती है।
- Excitation मे atom से electron का ह्रास नही होता है, बल्कि इलेक्ट्रान अपनी कम उर्जा वाले arbit से बाहरी arbit के अधिक उर्जा वाले arbit मे चले जाते है।
- Atomic particles की उपत्थिति के आधार पर particulate तथा non-particulate ionizing radiation में बाटा गया है।
- Non-particulate radiation, photon के बने होते है। तथा ये packets of energy होते है।
- ये photon जब किसी माध्यम से गुजरते है, तो अपने straight पथ पर energy deposit करते जाते है।
- Photon द्वारा अपने पथ पर प्रति इकाई लम्बाई पर energy की मात्रा जमा करता जाता है, उसे linear-energy transfer (LET) कहते है।
- जब radiation द्वारा अपने पथ पर प्रति micron दूरी पर प्रेरित आयनो के बिखरे होने के कारण उर्जा का जमाव कम करता है, तो LET का मान कम होता है, उसे sparsely ionizing radiation कहते है। जैसे- X-rays, gamma-rays.
- इसके अलावा, Radiation द्वारा अपने पथ पर प्रति micron दूरी पर प्रेरित आयनो (induce ion) के अधिक बिखरे होने के कारण energy का जमाव अधिक होता है, तो उसे densely ionizing radiation कहते है। जैसे- α-particles, Fast/Thermalneutrons.

(a) Particulate radiation

1. Alpha- rays (α):

- They are made of 2 protons and 2 neutrons and have double positive charge. They are densely ionizing, but lesser penetrating power.
- They are produced by fission of radioactive isotopes of heavier elements and they move in straight line.
- They have strong attraction for electrons and pull them away from the nuclei of atoms in their path and produce both ionization and excitation.
- After losing energy, each Alpha-particles captures two electrons and produces an atom of helium.
- As the alpha-particles move away from their source and produce densely ionization resulting chromosomal mutation.

2. Beta-rays (β):

- Beta-particles are high energy electron and produce from radioactive decay of heavier elements such as 3H, 32H, 35S, etc.
- They are sparsely ionizing but more penetrating power than Alpha-rays, they are negatively charged.
- Therefore, their action is reduced by positive charge of tissues.
- Electrons are easily defected by atoms in their path, hence they move in a zig-zag line.
- After their energy is spent, electrons attach to an atom making it negatively charged.
- Beta-rays may interact with the nuclei of atoms to produce electromagnetic radiation, and result in both chromosomal and gene mutation.

3. Fast and thermal neutrons:

- They are produced from radioactive decay of heavier elements in atomic reactors or cyclotrons.
- The high velocity of fast neutrons is produced by graphite or heavy water to generate thermal or slow neutrons.
- They are not repelled by nuclei of atoms, and move in a straight line
- Fast and thermal neutron do not cause ionization directly. Ionization is produced by-Elastic scattering in which nuclei of atoms are kicked away by the neutron, these nuclei then cause ionization.
- Production of gamma-rays- the thermal neutrons are captured by atomic nuclei which them become unstable and give off gamma rays.
- They are densely ionizing radiation and resulting in both chromosomal and gene mutation.

(b) non-particulate radiation (Electromagnetic radiation)

1. X-rays:

- X-rays were first discovered by Roentgen (1895).
- X-rays are electromagnetic radiation with wavelength of 10^{-11} - 10^{-7} $(0.001 - 10\text{Å})$.
- They are sparsely ionizing and heavy penetrating power. It may be produce in X-rays tubes.
- These are high energy radiation and consists photons, i.e., small packet of energy.
- They are commonly use for chromosomal breakage and produces all types of mutation in nucleotides such as addition, deletion, inversion, transposition, transitions, transversion, etc.
- These changes are brought out by adding oxygen to deoxyribose, removing amino or hydroxyl group and forming peroxides.
- X-rays include mutation by forming free radical and ions.

2. Gamma-rays (γ):

- They are also electromagnetic radiation with shorter wavelength and more penetrating power than X-rays.
- They are produce from radioactive decay of some elements like C^{14}, C^{60} $(cobalt\ 60), radium, etc$. The cobalt 60 is commonly used for the production of gamma rays.
- The injecting of gamma rays are pass through atom of tissues and cause chromosomal and gene mutation.

2. Non-ionizing radiation: Ultraviolet radiation

- They are produced by mercury vapour lamps or tubes. It is also present in solar radiation.
- UV rays have a wavelength of 100- 3900Å (10-390 nm), it can penetrate one or two cell layers.
- They are generally produce dimers of thymine, cytosine, etc. and some times cytosine present in the same strand of DNA. It also produces addition of a molecule of water to the double bond between 4 and 5 carbons of cytosine and uracil, which promotes deamination (dimer) of cytosine.
- The most effective wavelength of UV rays is 2540Å, since DNA bases show the maximum absorption at this wavelength.
- They are commonly used for radiation of microorganism like bacteria and viruses.
- In higher organisms, their use is generally limited to radiation of pollens in plants and egg in drosophila.
- UV rays can also break chromosome.
- In plant, pollen grains may be radiated and use for pollination, but the difficulty in collecting large quantities of pollen grains in most of the crop species except in maize and similar crop, and the limited duration of pollen viability have prevented the use of UV in crop improvement.

CHEMICAL MUTAGENS

1. Alkylating agents:

- They induce mutation by adding alkyl group (either ethyl (-CH3) or methyl (CH3-CH2) at various position in DNA. These group produces mutation by changing hydrogen bond and resulting transition and transversion.
- Transversion can occur either because a purine has been so reduced in size that it can accept another purine for it complement, or because a pyrimidine has been so increased in size that it can accept another pyrimidine for it complement.
- Alkylating agents includes EMS, MMS, EI, sulphur mustard, nitrogen mustard, etc.

2. Base analogues: such as 5-BU, 2-AP, 5-CU (chlorouracil), 5-IU (iodouracil)

- Base analogues are similar to bases of DNA which are add in DNA during replication.
- Such chemicals are added in DNA in place of normal base during replication. Thus they can cause mutation by wrong bases pairing resulting transition and transversion after DNA replication.
- 5 bromo uracil is similar to thymine but it has bromine at the C5 position, whereas thymine of bromine in 5BU enhances its tautomeric shift. Tautomeric change take place in all the four DNA bases (A, G, T and C), but at a very low frequency. The change or shift of hydrogen atom from one position to another either in a purine or in a pyrimidine base, is known as tautomeric shift, and such process is known as tautomerization.
- As a result, keto group (=O) of T and G is changed to enol group (-OH). Similarly, the amino group (-NH) of C and A is converted into amino group (-NH).
- Adenine (A) tautomer of 5 BU will pair with guanine (G) rather than with adenine(A). It will change to keto form at the time of DNA replication which will pair with adenine in place of guanine. As a result, AT→GC and GC→AT transition.
- The mutagen 2 AP (2 amino purine) acts in a similar way and cause AT↔GC transition. This is an analogue of adenine.

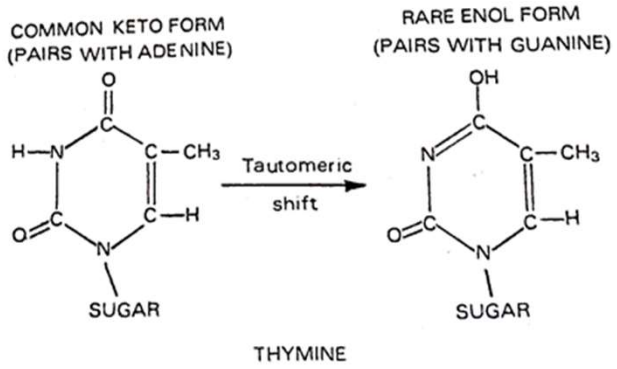
3. Acridine dyes:

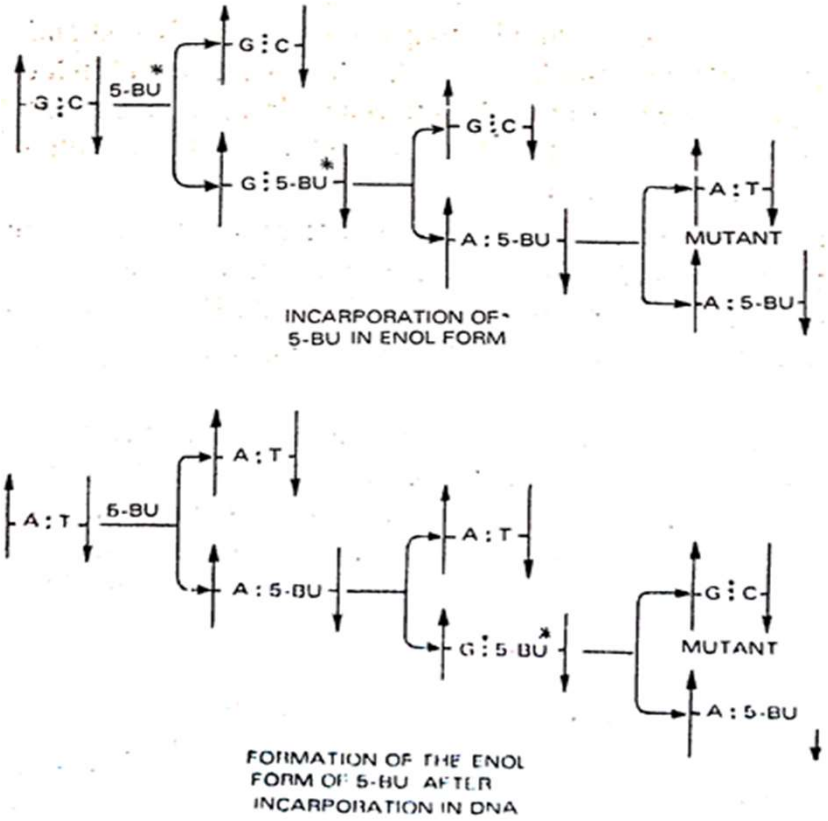
- They are very effective mutagens
- Proflavin and acriflavine are in common use for induction of mutation. They get inserted between two base pairs of DNA and lead to addition and deletion of single of few base pairs when DNA replicates and they cause frameshift mutations. They are also called frameshift mutagens.
- Proflavin is generally used for induction of mutation in bacteriophages.
- Acriflavin is used for induction of mutation in bacteria and higher organisms.

4. Other mutagens:

- Nitrus acid is power full mutagen which reacts with C6 amino group of cytosine and adenine. It replaced the amino group with oxygen (+H to –OH bond). As a result, cytosine act like thymine and adenine like guanine. This transition from GC↔AT are induced.
- Hydroxylamine is a very useful mutagen because it appears to be very specific and produces only one kind of change such as GC↔AT transition.

Tautomerization





ClB METHOD OF DROSOPHILA

The ClB technique was first developed by Muller (1927) which is based on mutagenic action of X-rays.

- In ClB chromosome, a special X chromosome has inverted region for crossover suppressor C in which crossing over is not done. A lethal gene l and a dominant gene B (responsible for bar eye) presented in the inverted segment.
- All ClB Females, identified by the bar eye shape, are heterozygous for this chromosome, but males having the ClB chromosome do not survive due to lethal l gene.
- In this method, males are irradiated with any chemical mutagen for the detection of sex-linked recessive lethal mutation.
- The treated males are crossed with ClB females.
- In the F1, half of the females will have the ClB chromosome which identified by the bar eye shape, the remaining half of the females will not have the ClB chromosome and are rejected.
- All the surviving F1 males will have the normal chromosome from the ClB females, while such males receiving the ClB chromosome will die due to the lethal l gene.
- Of the F1 progenies, the ClB female (bar eye) is mated with a normal eye male, all the progeny from such mating are kept in separate culture bottles.
- In the cultured progenies, the ClB female will have one ClB chromosome and one X chromosome from the mutagen treated male parent and are rejected.
- Half of the culture male progeny will receive the ClB chromosome and will die due to the lethal l gene. The remaining male progeny will get their X chromosome from their mutagen treated grandfather, if a lethal mutation was induced by the mutagen in this chromosome, these males will also die.
- Thus if the normal X chromosome of an F1 ClB female had a lethal mutation, there would be no male in it progeny.
- This technique is simple, rapid and there is little chance of an error in scoring. However, it is suitable for the scoring of sex-linked recessive lethal only.