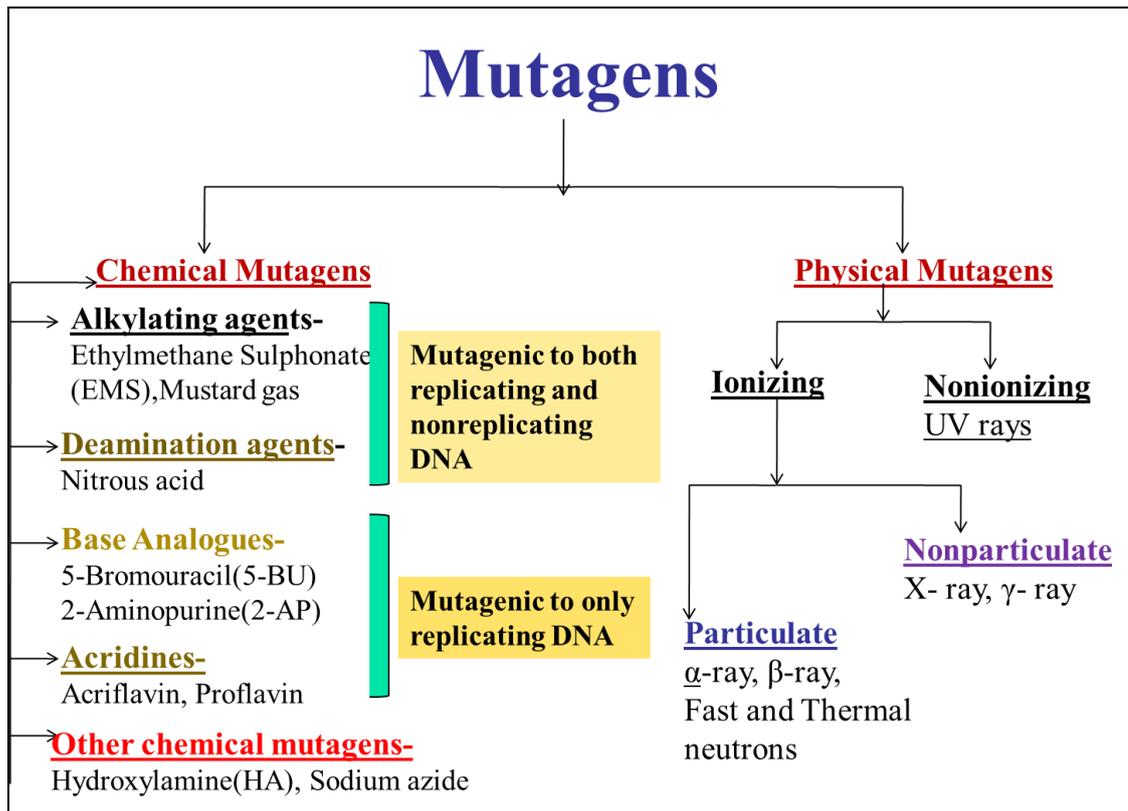


12. Mutagens

Artificial induction of mutations: Mutations can be induced artificially using

1. Physical mutagens or radiations
2. Chemical agents

Classification of mutagen



1. **Physical mutagens:** Include various types of radiations, viz., x-rays, γ -rays, α -rays, β -rays, fast neutrons, thermal or slow neutrons, UV rays etc. The physical mutagens are classified into
 - a) **Ionizing radiations:** They work through the release of ions. They have deep penetrating capacity. Eg: x-rays, γ -rays, α -particles etc.
 - b) **Non-ionizing radiations:** They function through excitation and have a very low penetrating capacity. They are used for studies on bacteria and viruses. Eg. UV rays.

Sources of physical mutagens:

- Gamma garden
- Gamma green house
- Vertical gamma irradiation facility
- Horizontal gamma irradiation facility
- X-ray machine
- Isotopes
- Small portable irradiators, accelerators and cyclotrons
- Nuclear reactors

2. **Chemical mutagens:** These can be divided into four groups.
 - a) **Alkylating agents:** This is the most powerful group of mutagens. These are the chemicals which are mainly used to induce mutations in cultivated plants. They induce mutations especially transitions and transversions by adding an alkyl group (either ethyl or methyl) at various positions in DNA. Alkylation produces mutation by changing hydrogen bonding in various ways. Eg: Dimethyl sulphonate (DMS), Ethyl methane sulphonate (EMS), Nitrosomethyl Urea (NMU), Nitrosoethyl Urea (NEU), Methyl methane sulphonate (MMS).
 - b) **Base analogues:** These are chemicals which are very similar to DNA bases, such chemicals are sometimes incorporated in DNA in place of normal bases during replication. Thus, they can cause mutation by wrong base pairing. An incorrect base pairing results in transitions or transversions after DNA replication. Eg: 5-bromouracil, 3-bromodeoxy uridine, 2-amino purine.
 - c) **Antibiotics:** A number of antibiotics like mitomycin and streptomycin have been found to possess chromosome breaking properties. Their usefulness for practical purposes is very limited.

- d) **Acridine dyes:** Acridine dyes Eg: proflavin, acriflavin, acridine orange, etc. are very effective mutagens. These are positively charged and they insert themselves between two base pairs of DNA. This is known as intercalation. Replication of intercalated DNA molecules results in addition or deletion of one or few base pairs which produces frame shift mutations.
- e) **Deaminating agents:** deaminating agents has strong mutagenic activity in a variety of viruses and microorganisms. But not useful in higher plants.
- f) **Miscellaneous:** Hydroxyl amine produce chromosomal aberrations. Nitrous acid.

Materials used for treating with mutagens:

Seeds, pollen, vegetative buds, whole plants, bulbils, tubers, suckers etc.

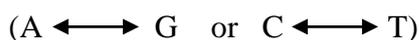
Molecular basis of mutations:

The term mutation is presently used to cover only those changes which alter the chemical structure of the gene at molecular level. Such changes are commonly referred to as “point mutations”. Point mutations involve a change in the base sequence of a gene which results in the production of a mutant phenotype. Point mutations can be subdivided into the following three classes based on molecular change associated with them.

1. Base substitution
2. Base deletion
3. Base addition

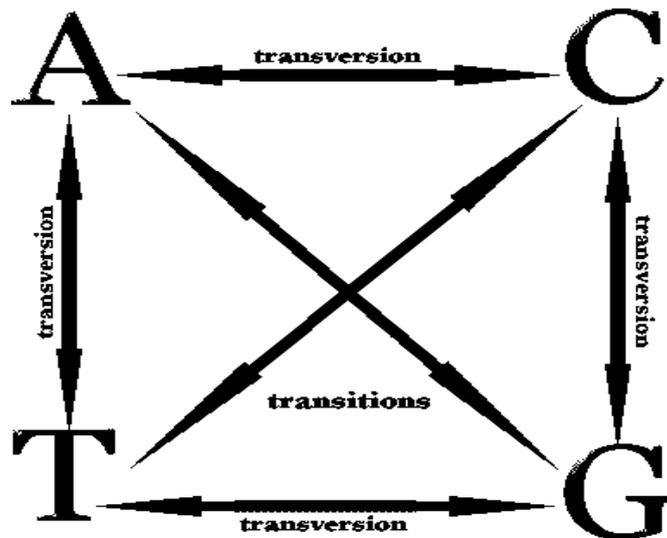
1. Base substitution: When a single base in a DNA molecule is replaced by another base it is known as base substitution (see Fig.). This can be of two types.

a) **Transition:** Replacement of a purine by another purine or a pyrimidine by another pyrimidine. (or) The substitution of a purine by another purine or of a pyrimidine by another pyrimidine base in DNA or RNA is known as transition.



b) **Transversion:** Replacement of a purine by a pyrimidine and vice versa. (or) The substitution of a purine by a pyrimidine or of a pyrimidine by a purine in DNA or RNA is known as transversion.





A base substitution may cause Neutral, Sense (silent), Missense or Nonsense mutation (see Fig.)

- A. **Neutral mutation:** A change in a base pair results in an amino acid change but the new amino acid has the same chemical properties as the old amino acid.
- B. **Sense (silent) mutation:** when one nucleotide is changed to another nucleotide but it doesn't affect the amino acid (protein) the gene codes for. In other words a change in base doesn't significantly alter the phenotype of the organism in which they occur.
- C. **Missense mutation:** change in a base produced altered protein.
- D. **Nonsense mutation:** change in one base leads results in a premature stop codon, or a nonsense codon which yield incomplete, and usually non-functional protein product

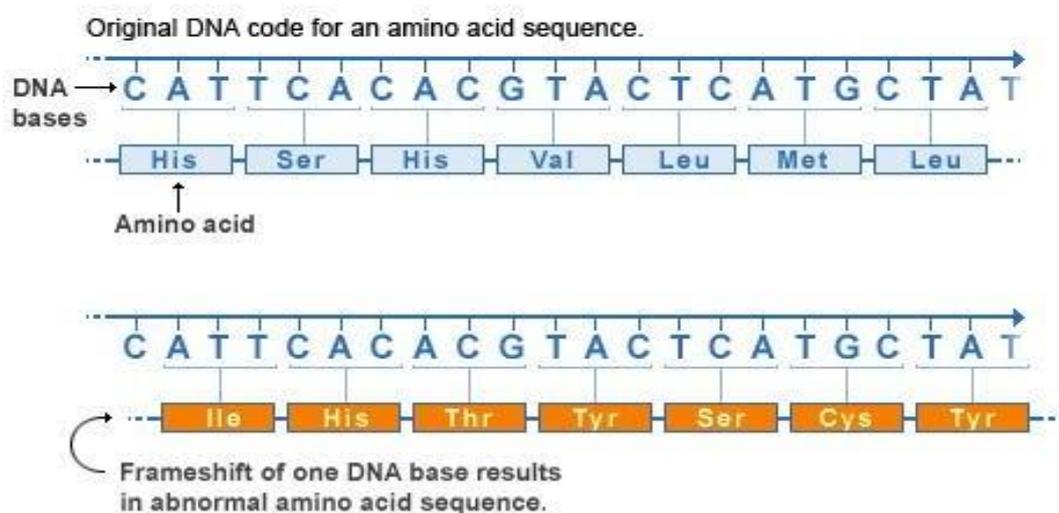
normal	AUG	GCC	TGC	AAA	CGC	TGG
	met	ala	cys	lys	arg	trp
		↓				
silent	AUG	GCT	TGC	AAA	CGC	TGG
	met	ala	cys	lys	arg	trp
			↓			
nonsense	AUG	GCC	TGA	AAA	CGC	TGG
	met	ala	---	---	---	---
			↓			
missense	AUG	GCC	GGC	AAA	CGC	TGG
	met	ala	arg	lys	arg	trp

2. **Base deletion:** In base deletion, one or more bases are altogether deleted
3. **Base addition:** There is insertion of one or more bases.

Addition or loss of bases in multiple of three, added or delete one to several amino acids from concerned protein, which may or may not give profound effect on activity of polypeptide. But if the number of bases added or deleted is not a multiple of three, a frameshift mutation is obtained, as the reading frame in such case is shifted from the point of addition or deletion onwards. Hence, in a frameshift mutation, all the amino acids of a polypeptide chain located beyond the site of mutation are substituted / altered.

Frameshift mutations: The mutations which arise due to addition or deletion of nucleotides in mRNA are known as frameshift mutations, because the reading frame of base triplets (codons) beyond the point of addition or deletion is altered as a consequence of such mutations (see Fig.).

Frameshift mutation



Detection of sex linked lethal mutations in Drosophila by Muller's CIB technique

Muller developed a system, CIB technique for detecting recessive sex-linked lethal mutations induced by X-ray treatment in Drosophila. He used a heterozygous (CIB) stock of Drosophila, which has a special X-chromosome, a large part of which is inverted (paracentric inversion). This acts as a crossover suppressor in the inverted region and is designated by C. A recessive lethal (l) gene and the dominant gene for bar (B) eye are located within the inverted segment, as a

result, the l and B are always inherited together in the same chromosome. The other X-chromosome was normal. Consequently, all CIB females identified by the bar eye shape, are heterozygous for this chromosome, while the males having the CIB chromosome do not survive [because of the l (lethal) gene].

The male flies were irradiated with X-rays for the induction of sex-linked recessive lethal mutations. Such males are crossed with CIB females. In the F₁ half of the females will have the CIB chromosome, which are easily identified by the bar-shaped eyes. The remaining half of the females will not have the CIB chromosome and are rejected. All the surviving F₁ males will have the normal chromosome from the CIB females, while those receiving the CIB chromosome will die due to the lethal gene 'l'. Each F₁ CIB females is mated to a normal male. Progeny from each such mating is kept in separate culture bottles. Each F₁ CIB female will have one CIB X-chromosome and one X-chromosome from the mutagen treated male parent. So, half of the male progeny receiving CIB X-chromosome will die. The remaining half male progeny will receive their X-chromosome from their mutagen treated grandfather which may or may not carry the induced mutation. In case lethal mutation was induced, no males will be observed. On the other hand, if no lethal mutation was induced, half of the males will survive. Thus, the CIB method was the simple, rapid and most efficient method for detecting sex-linked lethal mutations.

Detection of mutations in plants:

Generally, the seeds of a variety or strain are treated with the mutagens and grown to obtain M₁ plants. The M₁ plants are selfed to avoid out crossing due to partial male sterility in M₁ plants. The seeds thus obtained represent the M₂ generation. The M₂ plants are grown and the plants having mutant features are counted. Then the frequency of a given mutation is estimated as per cent ratio between the number of plants exhibiting a mutant phenotype in M₂ and the total number of plants in M₂.

$$\text{Mutation frequency (\%)} = \frac{\text{No. of plants exhibiting mutant phenotype in M}_2}{\text{Total number of plants in M}_2} \times 100$$

Significance of mutations in Plant Breeding:

1. When a variety is exceptionally good except for one or few characters.

2. When a recessive character is desirable and transfer of that character from wild species is difficult.
3. When a desirable character is linked with an undesirable character.
4. If there is no known source of resistance gene in the available germplasm
4. To create variability
5. To develop male sterile lines
6. To create variations in vegetatively propagated plants

Chimeras

Chimera is defined as a mixture of genetically diverse tissues in the same shoot. These tissues frequently form mosaic pattern. The most common and easily observed chimeras are leaf variegations observed in horticultural plants like Codium and Acalypha. The leaf variegations or chlorophyll variegations are due to plastid mutations, consisting of green and white or green and yellow patterning as also the patterning of anthocyanin distribution. These chimeras can be of three types.

1. **Sectorial:** The tissues possess the characteristics which closely resemble the parents from which these tissues are originally derived.
2. **Periclinal:** The core is of one plant and the epidermal region is of another plant. As a result, the leaves may be of one plant type and the flowers and fruits are of another plant.
3. **Chromosomal chimera:** The most frequent being the ploid-chimera. It has been a very common experience to meet with cells especially in the root tips, with higher chromosomal number in an otherwise diploid tissue. Plants under experimental control have been sometimes observed to give rise suddenly to sectors or whole branches differing in chromosome number.

Xenia

Effect of the genotype of pollen grain on the phenotype of seed tissues (embryo and endosperm) or the genetic effect of pollen parent upon the embryo and endosperm of seeds in some plants.