26. Mutation Breeding

Mutation is a sudden heritable change in a characteristic of an organisam. This definition requires that the change in the characteristic be heritable, but it does not state the genetic basis of the heritable change. Clearly, a mutation (as defined above) may be the result of a change in a gene, a change in chromosome(s) that involves several genes or a change in a plasmagene (genes present in the cytoplasm, *e.g.*, in chloroplasts, mitochondria, etc., which have circular naked DNA as chromosomes). 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 mutations.

Gene mutations can be easily and clearly shown by fine genetic analysis techniques available with microorganisms. Some mutations may be produced by changes in chromosome structure, or even in chromosome number; they are termed as chromosomal mutations. Gross chromosomal changes, *e.g.*, changes in chromosome number, translocations, inversions, large deletions and duplications are detectable cytologically under the microscope. But small deletions and duplications can rarely be detected, and would be considered as gene mutations.

This is particularly so in higher organisms where the techniques of genetic analysis are not yet as refined as those in the case of microorganisms. Thus what we refer to as gene mutation in plants is likely to include a fair number of small chromosomal changes. In clonal crops, mutations may even include gross changes in chromosome structure, sometimes even in number, unless cytological analyses are performed. Therefore, in this chapter, the word mutation would be used without a reference to the change in gene or chromosome (but easily detectable chromosome changes are not included) because in most of the cases the site of change is not known. When the mutant character shows cytoplasmic or extranuclear inheritance, it is known as cytoplasmic or plasmagene mutation. Another term bud mutation or somatic mutation, is used to denote mutations occurring in buds or somatic tissues, which are used for propagation, e.g., in clonal crops.

SPONTANEOUS AND INDUCED MUTATIONS

Mutations occur in natural populations (without any treatment by man) at a low rate; these are known as spontaneous mutations. The frequency of spontaneous mutations is generally one in 10 lacs,i.e., 10^{-6} but different genes may show considerably different mutation rates. For example, R locus in maize mutates at a frequency of 4.92×10^{-4}, Su at 2.4×10^{-6}, while Wx appears to be highly stable. Spontaneous mutation rates of genes may be considerably affected by the genetic background; some mutator genes may promote mutation of other genes. Mutations may be artificially induced by a treatment with certain physical or chemica1 agents; such mutations are known as induced mutations, and the agents used for producing them are termed as mutagens. The utilization of induced mutations for crop improvement is known as mutation breeding.

Mutation induction rarely produces new alleles; it produces alleles, which are already known to occur spontaneously or may be discovered if an extensive search were made. It is reasonable to say that induced mutations are comparable to spontaneous mutations in their effects and in the variability they produce. But the induced mutations have a great advantage over the spontaneous ones; they occur at a relatively higher frequency so that it is practical to work with them. Mutations have certain general characteristics; those that concern us the most are summarized below .

- Mutations are generally recessive but dominant mutations also occur.
- Mutations are generally harmful to the organism. Most of the mutations have deleterious effects, but a small proportion (Ca0.1%} of them are beneficial.
- Mutations are random. i,e., they may occur in any gene. However, some genes show higher mutation rates than others.
- Mutations are recurrent that is, the same mutation may occur again and again.
- Induced mutations commonly show pleiotropy, often due to mutations in c1osely linked genes.

Mutagens

Agents that induce mutations are known as mutagens. Mutagens may be different kinds of radiation (physical mutagens) or certain chemicals (chemical mutagens). The different mutagens may be grouped as follows.

A. Physical mutagens (all of them are various kinds of radiation)

1. Ionizing radiation

a. Particulate radiation. Eg. α – rays (DI), β- rays (SI), fast neutrons* (DI), and thermal neutrons (DI)

b. Non Particulate radiation (electromagnetic radiation), eg., X- rays* (SI), and γ- rays (SI).

2. Non Ionizing radiation. Eg. UV radiation.

B. Chemical mutagens

1. Alkylating agents, e.g., sulphur mustards, nitrogen mustards, epoxides, imines, (e.g., ethylene imine or EI)*, sulphates and sulphonates, diazoalkanes, nitroso compounds, e.g., N-methyl- N- nitro-N-nitroso-guanidine or MNNG).

2. Acridine dyes, e.g., acriflavine, proflavine, acridine orange, acridine yellow, ethidium bromide.

3. Base analogues, e.g., 5- bromouracil, 5-chlorouracil.

4.others, e.g., nitrous acid, hydroxyl amine, sodium azide*.

(* denotes that these agents are commonly used in mutation breeding. DI denotes densely ionizing and SI denotes sparsely ionizing radiations.)

Beta-Rays

Beta rays are high energy electrons produced by the decay of radioactive isotopes, e.g., ^{3}H, ^{32}P, ^{35}S, etc. High energy electrons are slowed down by positively charged molecules in and they have very little penetrating power as compared to X-rays. Beta-rays transfer energy to electrons of the atoms in their path causing these electrons to fly away from their orbit leaving the nucleus positively charged; this is known as ionisation. When the amount of energy transferred to an electron is not sufficient to cause ionisation, the electron is pushed to an outer orbit representing a higher level of energy, thus producing excitation. Electrons are easily deflected by atoms in their path, hence they move in a zig-zag line. After 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 similar to X-rays. Electrons liberated as a result of ionisation also produce ionisation and excitation, that is, they behave like beta-rays.

Alpha-Rays

The alpha particles making up alpha-rays have two protons and two neutrons each; thus the alpha-particles have double positive charge. Alpha-particles are produced by fission of radioactive isotopes of heavier elements. Since they are heavy particles, they move in a straight line. Alpha-particles have a strong attraction for electrons and pull them away from the nuclei of atoms in their path. Alpha-rays produce both ionisation and excitation. After energy, each alpha-particle captures two electrons and produces an atom of helium. As -particles move away from their source, they slow down and produce dense ionisation. Alpha particles are much less penetrating than neutrons and even beta-rays.

Fast and Thermal Neutrons

Fast Neutrons are produced in cyclotrons or atomic reactors as a result of radioactive decay of heavier elements. The velocity of fast neutrons is reduced by graphite or heavy water to generate thermal or slow neutrons. Neutrons are uncharged particles, and are highly penetrating in biological tissues. They are not repel1ed by nuclei of atoms, and move in a straight line. They do not cause ionisation directly. Ionisation is produced by (1) elastic scatering, in which nuclei, of atoms are kicked away by the neutron; these nuclei then cause ionisation, and (2) production of gamma-rays as thermal neutrons are captured by atomic nuclei, which then become unstable and give off gamma-rays. Fast and thermal neutrons are densely ionising radiations.

X-rays and Gamma Rays

X-rays and gamma-rays are nonparticulate electromagnetic radiation with a wavelength of 10^{-11} to 10^{-7} cm. These are high energy radiation and consist of photons, i.e., small packets of energy. The physical properties and the biological effects of X-rays and gamma-rays are similar, but they differ in the source of their origin. X-rays are produced by X-ray tubes, while gamma-rays are produced by radioactive decay of certain elements, e.g., radium, ^{14}C, ^{60}Co, etc. ^{60}Co is the common Source of gamma-rays used for biological studies. X-rays are often referred to as hard (0.1-0.001A°) or soft (10-1A°) depending upon their wavelength. X-rays and gamma-rays are highly penetrating and sparsely ionising. The electromagnetic radiations produce the following effects.

Photoelectric Effects

Low energy photons transfer all their energy to individual electrons, which are kicked off as high energy electrons (e-) from their orbit producing ionisation. These high energy e- produce secondary ionisations, which are of greater significance than the primary ones.

Compton Scattering

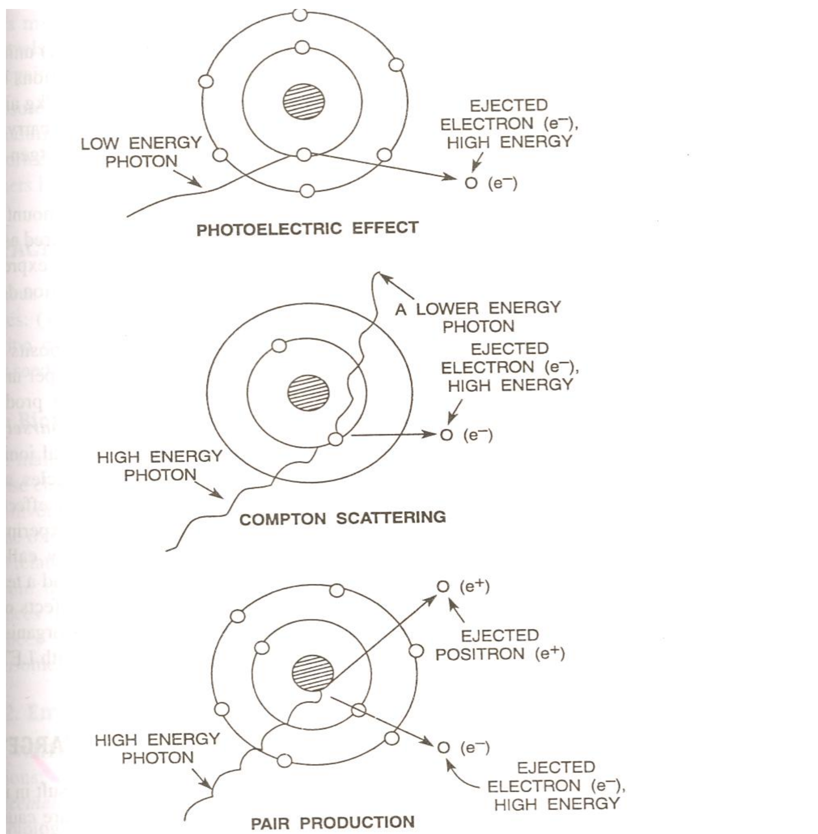
A high energy photon transfers a part of its energy in kicking away an e- of an atom from its orbit producing ionisation. The wavelength of such a photon becomes increasingly longer as it loses energy in repeated ionisations.

Pair Production

A high energy photon passing close to the nucleus of an atom may be completely absorbed accompanied with the ejection of a high energy e- and a high energy positron (e+); the high energy e- and e+ produce ionisation and excitation.

Ultraviolet Radiation

Ultraviolet (UV) rays have a wavelength of 100 to 3,900A° (10-390 nm). UV is present is solar radiation and is produced by mercury vapour lamps or tubes. UV is a low energy radiation; it does not cause ionisation and has a very limited penetrating capacity (usual1y limited to one or two cel1layers). UV rays generally produce dimers of thymine, uracil and, sometimes, cytosine present in the same strand of DNA. It also produces addition of a molecule of water to the 5, 6 double bond of uracil and cytosine, which promotes deamination of cytosine. The mutagenic action of UV is most likely due to dimer formation and deamination. The most effective wavelength of UV is 2,540A° since DNA bases show the maximum absorption at this wavelength.



Ion production by X-ray and gamma-ray photons. The large central solid circle represents the nucleus of an atom, the small open circles on the periphery denote electrons (e^-), and the particle e^+ is a positron. The atom pictured here is an oxygen atom.

UV is commonly used in microorganisams since penetration presents no problem in that system. But in higher organisams, poor penetration of UV has limited its use to irradiation of pollen grains (in plants) and of small eggs (e.g., in Drosophila). In plants, pollen grains may be irradiated and used for pollination. But the difficulty in collecting large quqntities of pollen grains in most of the crop species, except in maize and similar crops, and the limited duration of pollen viability have prevented the use of UV in crop improvement.