Subject - M.Sc Botany, (Sem-IV)

MBOTEC – 1, Paper : Cytogenetics and Crop Improvement

Topic – Mutation and its role in Crop Improvement (UNIT II)

Dr. Punam Ranjan

Assistant Professor

Department of Botany

Patna Science College, Patna.

e-mail gayatripunam@gmail.com

MUTATION

The term mutation was originally coined by Dutch **botanist Hugo De Vries** (1848–1935) to describe a new approach to explain evolution, although it is quite different than the current definition.

Definition: A sudden change in the genetic information is called a mutation. Thus the term **mutation** refers to both the change in the genetic material and the process by which the change occurs. An organism that exhibits a novel phenotype resulting from a mutation is called a **mutant**.

Every one of us has been shaped (has unique genetic makeup) due to some particular mutations. In fact, most people have dozens or even hundreds of mutations in their DNA. Mutations are essential for evolution to occur. They are the ultimate source of all new genetic material—new alleles in a species. Although most mutations have no effect on the organisms in which they occur, some mutations are beneficial. Even harmful mutations rarely cause drastic changes in organisms.

As per a most recent paper, PLOS Genetics Review on 1 April 2020 (https://doi.org/10.1371/journal.pgen.1007995) by **Devon M. Fitzgerald and Susan M. Rosenberg,** mutation is introduced as below-

"Mutation is any change in the sequence of an organism's genome or the process by which the changes occur. Mutations range from single-base pair alterations to mega base-pair deletions, insertions, duplications, and inversions. Though seemingly simple, ideas about mutation became entangled with the initially simplifying assumptions of both Darwin himself and the "Modern Synthesis"- the geneticists who embraced Darwin in the pre-DNA early 20th century, beginning evolutionary biology. The assumptions of purely "chance" mutations that occur constantly, gradually, and uniformly in genomes have underpinned

biology for almost a century but began as a "wait-and-see"- based acknowledgment by early evolutionary biologists that they did not know the chemical nature of genes or how mutations in genes might occur. Darwin considered generation of variation by chance to be a simplifying assumption, given that the origins of variation (genes) were unknown in his time."

Mutations are classified on many basis

On the basis of nature of occurrence:

- Spontaneous mutations are result of errors that occur naturally in DNA.
- Induced mutations are result of errors that occur by exposure to radiation, chemicals, viruses, or other mutagenic agents.

On the basis of cell type:

- Germline mutations occur in gametes. These mutations are especially significant because they can be transmitted to offspring and every cell in the offspring will have the mutation.
- Somatic mutations occur in other cells of the body. These mutations may have little effect on the organism because they are confined to just one cell and its daughter cells. Somatic mutations cannot be passed on to offspring.

On the basis of part/portion of genetic material affected:

- Chrosomal Aberration: Physical/Structural/Visible changes in chromosome that might be accidental or induced.
- **Point Mutation**: A point mutation is a change in a single nucleotide in DNA. This type of mutation is usually less serious than a chromosomal alteration. An example of a point mutation is a mutation that changes the codon UUU to the codon UCU. Point mutations can be divided into three types -

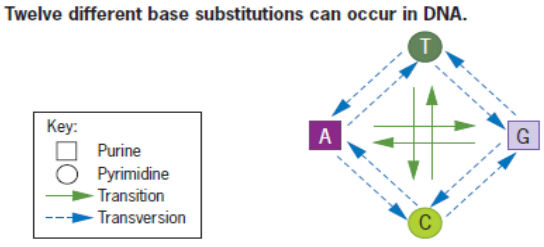
1. Silent mutation (mutated codons codes for the same amino acid, cause no effect)
2. Missense mutation (mutated codons codes for the different amino acid, cause variable effect)
3. Nonsense mutation (mutated codon is a premature stop codon, generally cause serious effect)

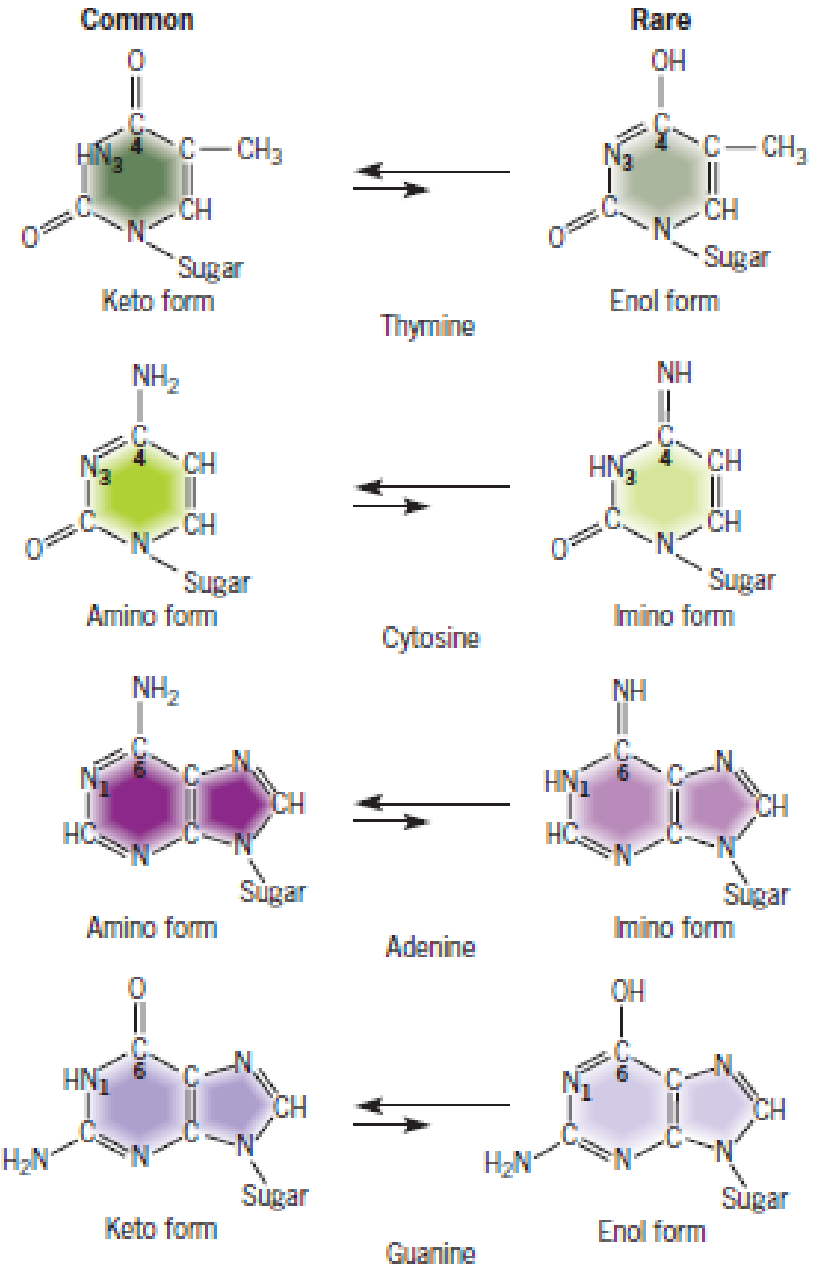
MOLECULAR MECHANISM OF MUTATION

When Watson and Crick described the double-helix structure of DNA and proposed its semiconservative replication based on specific base-pairing to account for the accurate transmission of genetic information from generation to generation, they also proposed a mechanism to explain mutation.

Watson and Crick pointed out that the structures of the bases in DNA are not static. Hydrogen atoms can move from one position in a purine or pyrimidine to another position for example, from an amino group to a ring nitrogen. Such chemical fluctuations are called **tautomeric shifts.**

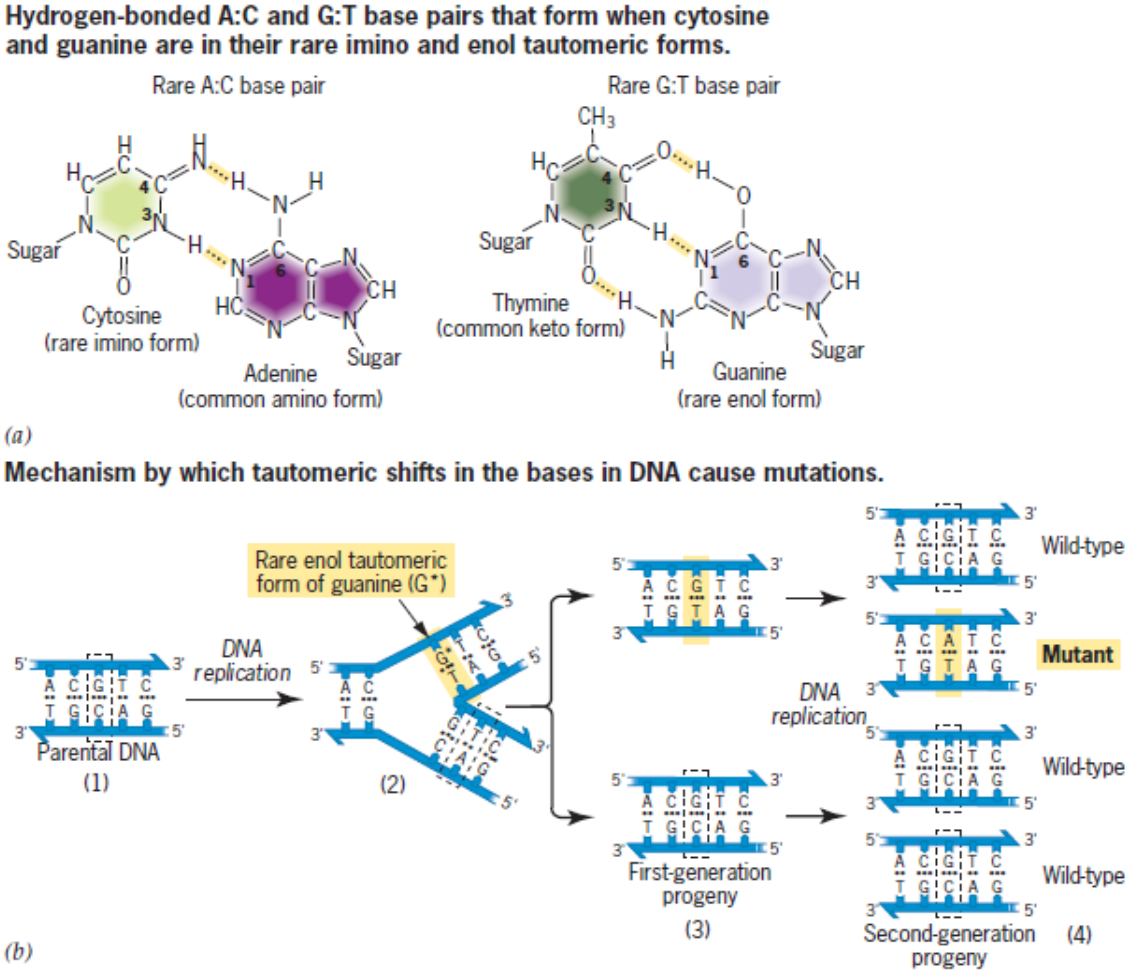
Base pair substitution: Mutations resulting from tautomeric shifts in the bases of DNA involve the replacement of a purine in one strand of DNA with the other purine and the replacement of a pyrimidine in the complementary strand with the other pyrimidine. Such base-pair substitutions are called **Transitions.** Base-pair substitutions involving the replacement of a purine with a pyrimidine and vice versa are called **Transversions.**





Tautomeric Shift for all four Bases

From Principles of Genetics 6th ed by Snustad and Simmons

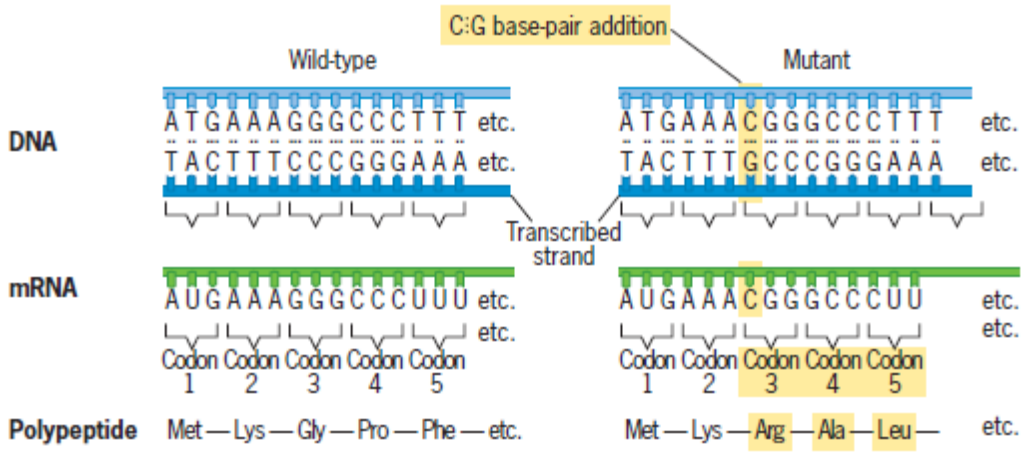


Mutation due to Tautomeric shift that causes Base pair substitution

From Principles of Genetics 6th ed by Snustad and Simmons

<u>Frameshift Mutation</u>: A frameshift mutation is a deletion or insertion of one or more nucleotides that changes the reading frame of the base sequence. Deletions remove nucleotides, and insertions add nucleotides.

All three types of point mutations **Transitions**, **Transversions**, and **Frameshift** mutations are present among spontaneously occurring mutations.



Frameshift Mutation

From Principles of Genetics 6th ed by Snustad and Simmons

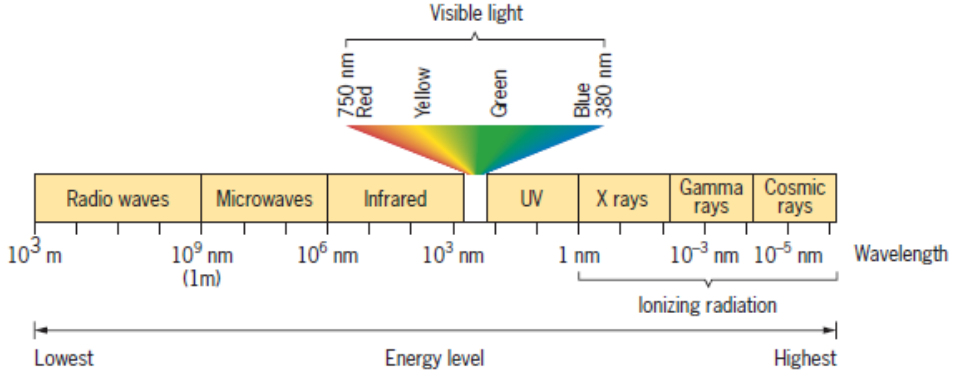
Types of Mutagens

In 1927 Hermann J. Muller discovered X-rays induced mutations in *Drosophila*.

Mutagens: Physical (Radiations) and Chemical

Mutagenesis: Mutations arising from (induced by) exposure to radiation or chemicals.

1. **Radiation mutagenesis**: mutations that arise from ionizing (X-rays, α, β, and gamma rays) or non-ionizing (UV light) radiation.

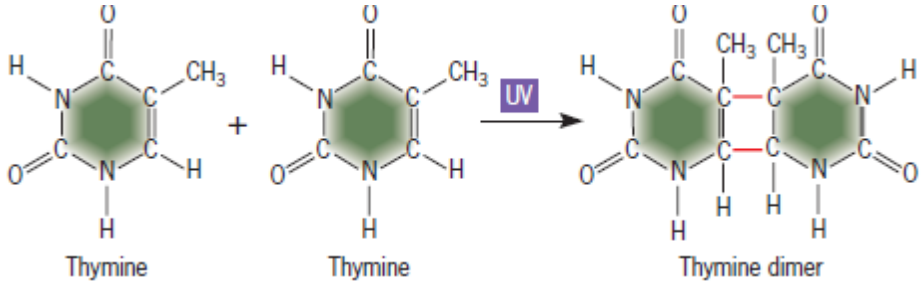


Electromagnetic spectrum

From Principles of Genetics 6th ed by Snustad and Simmons

Ionizing radiation: High energy, low wavelength. These Mutations arise directly from structural alterations to DNA, or indirectly from structural alterations of molecules (metabolites) that interact with DNA. Thus damage itself arises from ionization, are due to positively-charged free radicals result from release of electrons in target molecules. These structural alterations to DNA can be severe, including disruption of covalent linkages. Damaged regions can involve nitrogenous bases, the sugar moiety, and the phosphodiester backbone. Damage to DNA itself can cause a phenotypic effect, as DNA cannot be metabolized (e.g., replicated, transcribed, etc.) properly. DNA repair mechanisms can "fix" structural damage, but often do so incorrectly by removing the affected DNA but inserting an incorrect base.

UV radiation: Primary photoproducts (damage) of UV radiation are pyrimidine-pyrimidine dimers (mostly thymine-thymine dimers), that causes the reason for mutation. Other, less frequent UV photoproducts are pyrimidine hydrates. Similar to other radiation damage, DNA damaged by UV radiation must be repaired for DNA to be metabolized correctly or may lead to a heritable mutation. Xeroderma pigmentosum is a disease which is cused due to mutation induced by UV radiation.



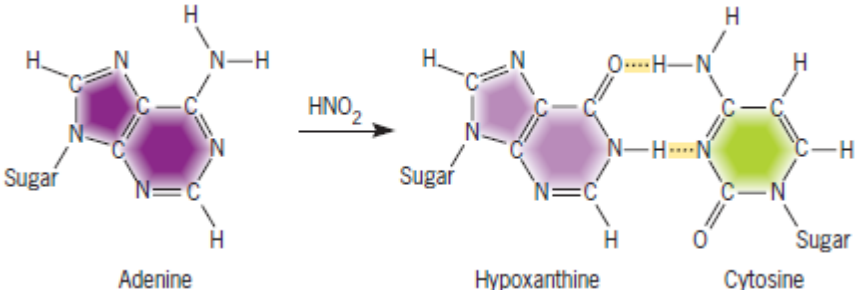
Cross linking of adjacent Thymidine molecule

From Principles of Genetics 6th ed by Snustad and Simmons

2. Chemical mutagenesis: Mutations that arise from exposure to certain chemicals. Chemical mutagens are generally of two types: those that are mutagenic to both replicating and non-replicating DNA (includes alkylating agents and deaminators), and those that are mutagenic to replicating DNA only (includes base analogs & acridine compounds).

Alkylating agents (e.g., EMS, MMS) are chemicals that transfer methyl- or ethyl- (alkyl-) groups onto nitrogenous bases, thus altering base-pairing potential. Perhaps the most cause of point mutations in humans is spontaneous addition of a methyl group ($-CH_3$). EMS, for example, causes ethylation (addition of a $-CH_2CH_3$ group) at the N-7 of guanine. This causes guanine to base pair with a thymine at the next round of DNA replication.
G≡C → G*=T → A=T [transition mutation]

Deaminators (e.g., nitrous acid, a red meat color preservative) are chemicals that deaminate (remove amine or $-NH_2$ groups)) from nitrogenous bases. Nitrous acid (HNO_2), for example, deaminates adenine to hypoxanthine.



Oxidative deamination of Adenine

From Principles of Genetics 6th ed by Snustad and Simmons

Thus Hypoxanthine will base pair with a cytosine at the next round of DNA replication.
A=T → hypoxanthine -T → hypoxanthine = C → G≡C [transition]

Base analogues (e.g., 5-bromouracil) are nitrogenous bases (or derivatives) that are not normally found in DNA, but which can be incorporated mistakenly during DNA replication. Most base analogs can have base-pairing relationships that differ from the base for which they were mistaken during DNA replication. 5-bromouracil in its normal state is mistaken for thymine during DNA replication. At a subsequent round of DNA replication, 5-bromouracil base can enter into a rare state, behave like a cytosine, and base pair with guanine, not adenine. This will lead to a G≡C base pair where there used to be an A=T base pair (transition). If 5-bromouracil shifts back to its normal state, it will base pair with adenine.

A=T → A – 5-BU → G ≡ 5-BU → G≡C [transition]

The reverse also can happen, where 5-bromouracil in its rare state can be incorporated during DNA replication in place of cytosine, shift to its normal state, and base pair with adenine at a subsequent round of replication. This will lead to A=T base pair where there used to be a G≡C base pair.

G≡C → G – 5-BU →A – 5-BU → A=T [transition]

Acridine compounds are molecules that bind directly (intercalate) into DNA, resulting in additions/deletions of one of a few bases. Acridines cause frameshift mutations in protein- coding sequences (genes).

- All the point mutations (spontaneous/induced) are either due to base pair substitution (transition/transversion) or frameshift (insertion/deletion).
- A mutation in a wild-type gene can produce a mutant allele that results in an abnormal phenotype. However, the mutant allele can also mutate back to a form that restores the wild-type phenotype. Therefore **mutation is a reversible process**.

ROLE OF MUTATION IN CROP IMPROVEMENT

Globally, the current human population is increasing day by day and expected to reach 9 billion by 2050 and that will lead to food scarcity on earth. To overcome this increasing demand for food and proper nourishment, an improvement in food production is urgently needed. Because of limited available arable land, depleting water resource and varying climatic condition, it becomes a major task. The difficulties are also compounded by urbanization, salinization, biotic stress, drought and desertification that result in a reduction of arable land. Moreover, changing climatic conditions and subsequent variations also limit food production.

There are different mechanisms for harnessing the heritable variations encoded in the genetic makeup of existing crop plants so as to use them in the crop improvement programs. Among the different strategies to enhance crop improvement programs, induced mutagenesis has contributed immensely by creating mutant varieties with improved and desirable genetic changes in agronomically important traits of the crop plants. Mutagenesis has become more efficient in combination with advanced molecular biology techniques and in vitro culture methods that result in enhancement of crop improvement/breeding program particularly under the global climate change. Such induced mutagenesis also helps in the mining of new gene alleles that do not occur in the germplasm.

Spontaneous mutation and crop improvement

Spontaneous mutations in crop plants occur naturally during adaptations and evolutionary processes at an extremely low rate i.e. 10-5-10-8. This frequency is in adequate for creating variations in the genetic architecture of a crop for improvement of desirable traits. Wheat, peas

and barley are the notable example of mutants derived through heritable permanent change i.e, spontaneous mutation during the course of domestication. Spontaneous mutation in these plants resulted in eradicated pod or head shattering and the reduction in seed dormancy periods. High yielding and lodging resistance in wheat varieties were developed by the incorporation of Spontaneously-mutated alleles of the genes that resulted in the green revolution and subsequently secured food for millions of people around the world. Other examples include utilization of dwarf germplasm Dee-geo-woo-gen from china and the release of rice variety IR8 developed in the Philippines by the International Rice Research Institute (IRRI) from a dwarf line.

Induced mutation and crop improvement

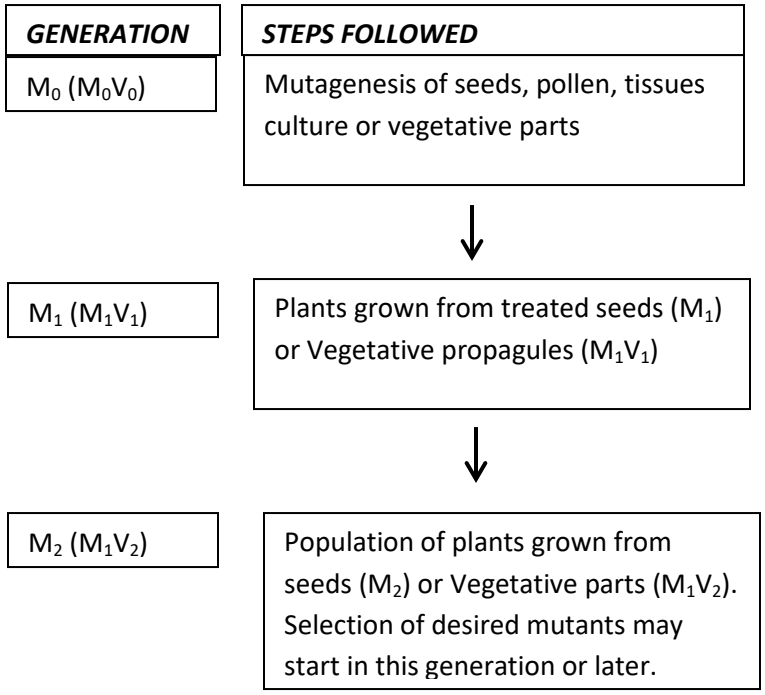
Since the 1920s, plant breeders have taken advantage of physical and chemical mutagenesis to introduce genetic variation. Some traits in crop species have been obtained from naturally or deliberately induced mutations in the same genes. For example, semi-dwarf varieties of rice that enabled the Green Revolution were derived independently from natural and induced mutations in the gene for gibberellin 20-oxidase. In hexaploid wheat, natural and induced mutations in waxy homologs have been combined by breeding to modify starch quality. Mutations were first induced in plants with physical mutagens and this methodology has produced the majority of the varieties (77 %) listed in the FAO/IAEA Mutant Varieties.

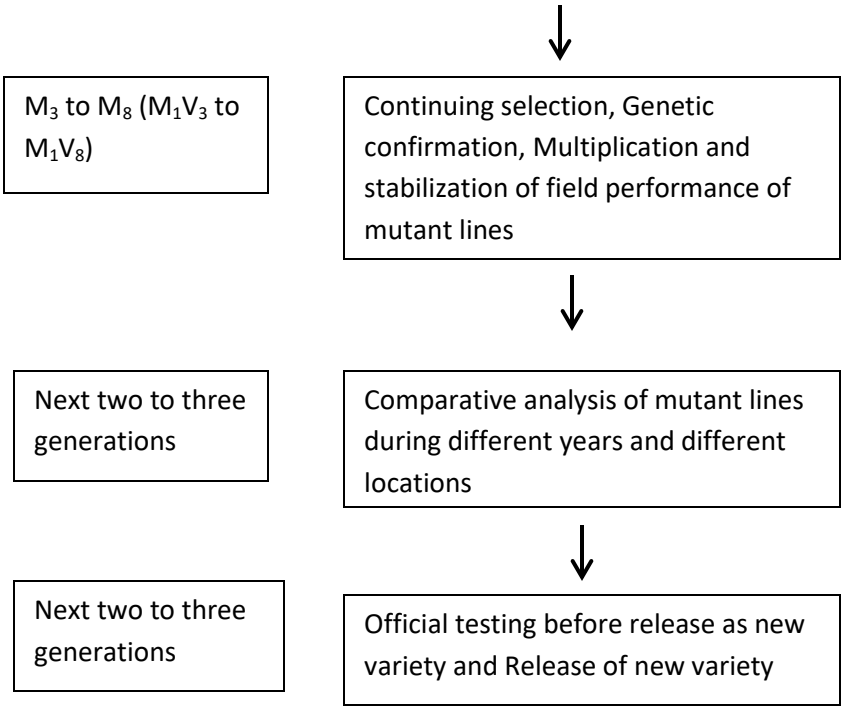
Physical mutagenesis is most commonly conducted with ionizing radiation produced directly by gamma and X-rays or indirectly by fast neutron bombardment. It is generally assumed that gamma radiation causes less chromosomal damage and more point mutations and short deletions compared to X-rays and fast neutrons. However, a genome wide analysis of mutations induced by fast neutron bombardment in *Arabidopsis thaliana* found a higher frequency of novel single nucleotide polymorphisms (SNPs) than deletion.

Studies on induced mutation in groundnut; *Arachis hypogea* were first carried out by X rays by some scientists. Few physical mutagens had been tested for groundnut mutagenesis during the past 50 years. From these, by physical mutagenesis it were obtained 65 varieties: 10 varieties (X-rays), 41 (gamma rays), 12 (beta rays) and 2 varieties with laser influence. Physical mutagens include radiations such as α-rays, β-rays, fast neutrons, thermal neutrons, X-rays, Y-rays, and ultraviolet radiation.

Chemical mutagenesis agents was attempted by many people over a long period, but there were no clear or convincing positive results until 1939 when Thom and Steinberger found that nitrous acid was effective in causing mutations in Aspergillus. Now Chemical mutagenesis has become a widely adopted approach because it does not require special facilities and the resulting mutations are primarily SNPs. Ethyl methane sulfonate (EMS) is currently the most commonly used chemical mutagen, but methyl-nitrosourea, sodium azide, diethyl sulfate and di-epoxy-butane have also proven effective.

Steps in Traditional Mutation Breeding





Mutation breeding in India for crop improvement

Mutation breeding in India has many achievements, related to crop improvement and for developing more than 345 improved mutant varieties belonging to 57 crop species. Largest number of mutant varieties (70 per cent) have been induced by physical mutagens (gamma rays) being the most commonly used and also found to be highly successful.

The four high yielding chickpea mutant varieties, Pusa-408, Pusa-413, Pusa-417, and Pusa-547 developed by the author at the Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi and released by the Government of India for commercial cultivation are the first ever examples of direct use of induced micro-mutants in a legume crop in the world.

Rice variety PNR-102 and PNR-381 have very high economic impact.

The latest chickpea mutant variety Pusa-547, released in 2006 for cultivation has attractive bold seeds, thin testa and good cooking quality. Similarly several mutant varieties of groundnut and black gram variety released by Bhabha Atomic Research Centre (BARC), Mumbai and cultivated in millions of acres in Maharashtra, Gujarat and several other states, have contributed tremendously towards achieving the targets of the agricultural production and also to the Indian agricultural economy as a whole.

Suggested Books and References

1. Principles of Genetics 6th Edition by Snustad and Simmons.
2. Cell and Molecular Biology 7th Edition by Gerald Karp.
3. Plant Breeding by B. D. Singh.
4. Devon M. Fitzgerald and Susan M. Rosenberg, What is mutation? A chapter in the series: How microbes "jeopardize" the modern synthesis. PLOS Genetics Review, April 2020. https://doi.org/10.1371/journal.pgen.1007995.
5. Mohammedsani Zakir. Mutation Breeding and its Application in Crop Improvement under Current Environmental Situations for Biotic and Abiotic Stresses. IJRSAS. Volume 4, Issue 4, 2018, PP 1-10. http://dx.doi.org/10.20431/2454-6224.0404001.
6. Shashikala S Kolakar et, al. Role of mutation breeding in improvement of medicinal and aromatic crops: Review. Journal of Pharmacognosy and Phytochemistry 2018; SP3: 425-429.
7. https://www.osti.gov/etdeweb/servlets/purl/608640 (For historical details of Mutation breeding for disease resistance)
8. Deepak Pental. When scientists turn against science: exceptionally flawed analysis of plant breeding technologie. CGMCP, DU, South Campus, REVIEW ARTICLE 932 CURRENT SCIENCE, VOL. 117, NO. 6, 25 SEPTEMBER 2019. (For a comparative analysis of techniques)

- ✓ Please go through the References 3, 5 and 6 for more crops examples in Mutation breeding.