**Mutation breeding in crop improvement**

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**ABSTRCT**

Genetic variation is the backbone, which plant breeding requires producing novel and improved crop cultivars. Providentially, mutation breeding gives us hope to generate food crops that are high-quality in yield, nutritionally enhanced to improve the content as well as the bio-availability of essential nutrients. They also produce crop varieties that are disease resistant, drought resistant and salt tolerant. As the role and profusion of variations of transgenic crop in human food systems and their effect on ecology, human health and agriculture biodiversity is better understood and well documented but the role of mutagenic plants and their role in human food systems is less understood.

Ever since the discovery of mutation effects of X-rays, the mutation breeding has proved to be a potential and unparalleled technique for the improvement of crops. New and advanced techniques in the mutation induction though have come up but every time they have served the basic cause of the crop improvement for providing the sustainable nutrition, food security, enhancing the nutritional quality and to help the ever increasing demands of population .The heritable variability caused by the mutations also helps to overcome the general decline in the genetic diversity that has been occurring continuously in the crop species.

**Keywords**: mutation breeding, mutagenesis, radiations, transition and etc.

**I. Introduction**

A mutation is a sudden, heritable change in an organism's trait. These modifications are the results of chemical changes made to the genes. Such modifications can result in novel and heritable character changes in agricultural plants and these variations can be selected and applied to the development of novel crop varieties. Rarely do mutations occur in the natural world. Spontaneous mutations are those kinds of mutations. However, certain chemical or physical substances known as mutagens or mutagenic agents can help to increase the frequency of mutations and mutations brought about in this way are known as induced mutations. By using such agents, crop plants can undergo mutations, and the best varieties that result from these mutations can be chosen. Mutation breeding is the process of developing new cultivars of plants with optimum characteristics with the use of induced mutations. Since Muller established in 1928 that exposure to X-rays can result in DNA changes in *drosophila melanogaster*, radiation has been proposed as a mutagen. Radiation has been extensively employed to create novel cultivars used for crop production and as genetic resources since Stadler first published papers on the mutations caused by irradiation in maize and barley. Radiation mutation breeding has unmatched benefits over other breeding techniques, including cross-breeding and chemical mutagenesis, thanks to its broad mutation spectrum and high mutation efficiency. More than 1,000 novel kinds have been used and promoted globally, and 3,365 mutant types have been recorded in the IAEA's Mutant Variety Database as of this writing. Caused mutations are modifications, additions, or deletions of nitrogen bases at the molecular level. They can be divided into transitions, transversions, and frame shift as a result. The term "transition mutation" refers to the substitution of one purine or pyrimidine with any other purine or pyrimidine. In a conversion mutation, a purine is switched out for a pyrimidine, or vice versa. Frame shift mutation, on the other hand, alters the gene's reading frame by adding or removing nitrogen bases. This is especially true for higher creatures, where genetic analysis techniques are still not as advanced as they are for bacteria. Consequently, the phenomenon known as gene mutation in plants is likely to be composed of a a good number of minor chromosomal alterations. If cytological tests are not done, mutations in clonal crops may even include significant changes in chromosome structure, occasionally even in number. Because the location of change is unknown in the majority of cases, the term "mutation" will be used in this chapter without referring to a change in a gene or chromosome (however easily visible chromosome changes are not covered). Cytoplasmic or plasma gene mutation is the term used when the mutant character exhibits cytoplasmic or extra nuclear inheritance. Another word for mutations occurring in buds or somatic tissues, which are employed for propagation, such as in clonal crops, is bud mutation or somatic mutation.

**II. History of plant mutagenesis**

According to some theories, reports of mutant crops in China about 300 BC could be used to date the beginning of plant mutation. English farmer Seth wright recorded first case of mutation in 1791 in male lamb with unusual short leg. The term mutation is coined by Hugo de vriesin 1900 by his observation in *oenothera lamarkiana* . Systematic study of mutation was started in 1910 when Morgen genetically analyzed white eye mutant of drosophila. Muller 1927 induces mutation in drosophila by using X ray. He was awarded with noble prize in 1946. Hugo de Vries, working on the "rediscovery" of Mendel's principles of heredity, was the first to recognize mutations as a method for producing diversity in the late nineteenth century. He viewed this diversity as heritable alterations caused by fundamentally different mechanisms than segregation and recombination. He defined this phenomenon as sudden, inherited alterations in organisms that have a major impact on their phenotypic appearance He described this occurrence as swift changes in organisms, which were hereditary and thus produced relatively large effects on the phenotypic appearance of organism After Stadler discovered the mutagenic activity of X-rays in maize, barley, and wheat, the field of radiation-induced mutations as a technique for creating unique genetic variety in plants advanced.

In 1934, the first commercial mutant tobacco strain was created. Acquaah reported 77 cultivars that were created using mutagenesis before 1995. There were 484 commercially available types in 1995, a rise. Since then, this number has significantly expanded as new mutant kinds are regularly reported across many continents. Fruit trees (mango, papaya, banana, etc.) and ornamentals (rose, gerbera, marigold, etc.) as well as food crops (chicken pea, wheat, green gram, etc.) are only a few examples of the plants. Agronomic features such as lodging resistance, early maturation, winter hardiness, and product quality have been altered as a result of mutant breeding.

**Why are we interested in Mutants?**

The ultimate source of variation is mutation. Some mutations lead to newer versions of proteins and help the organisms to adapt to changes in the environment. As the first step of evolution, the mutation is important as it creates a new DNA sequence for a specific gene which creates a new allele. For a specific gene via iatrogenic recombination, recombination could also create a new DNA sequence.

**III. Spontaneous mutation**

The sum of everything that might go wrong with DNA during an organism's life cycle is what causes spontaneous mutations, according to science (Glickman et al., 1986). Therefore, all mutagenic and anti-mutagenic biological mechanisms combine to produce the types and quantities of spontaneous mutations. The fact that new changes in experimental settings significantly alter the types and rates of spontaneous mutations is not commonly understood. All mutations, including base substitutions, frame shifts, insertions, and deletions, occur naturally. The studies of the mechanisms underlying spontaneous mutagenesis and the slight experimental variables that influence the types and frequencies of spontaneous mutations have, however, received very little attention in the literature. This is regrettable because spontaneous mutagenesis appears to be a major factor in cancer, ageing, and evolution. This review focuses on minute experimental factors that significantly influence a spontaneous mutation experiment's findings. A hypothesis of "directed" mutagenesis is not necessary once these factors are well understood. We examine the genetic regulation of spontaneous mutagenesis, the intrinsic instability of DNA, and the types of normal metabolic lesions formed in DNA that result in mutations via mistakes in replication, repair, and recombination. Similar to spontaneous mutagenesis, spontaneous carcinogenesis can be seen as the culmination of all possible DNA errors that can occur over an organism's lifetime. In the genome, spontaneous mutations happen naturally. They typically happen as a result of a replication, mitosis, meiosis, or other process mistake Transposons or mobile genomic elements can also cause mutations.

The frequency of spontaneous mutations is generally one in 10 lacs, i.e., 10-6.

Spontaneous mutations are primarily brought on by:

* Replication errors
* Slipped strand mispairing
* Wobble base pairing
* Depurination or deamination
* Tautomerism
* Unequal crossing over

**Examples of Spontaneous Mutations**

Spontaneous mutations may occur in bacteria, animals, plants, and humans. Some of the examples of spontaneous mutation include:

* Lactose fermentation character in Escherichia coli
* Radiation resistance trait in Escherichia coli
* Shrunken seeds, purple color, colorless or sugary traits in corn or maize plant.
* Sensitivity of Escherichia coli to streptomycin (antibiotic) trait.
* Fruit fly traits include yellow body, brown eyes, white eye, brown eye, or eyeless.
* Traits such as pink eyes, brown coat, or piebald coat in the house mouse.
* A wild animal born naturally with black eyes instead of typical green eyes.
* Hemophilia, Huntington disease, Retinoblastoma, Sickle-cell anemia, and Aniridia (eye disorder where iris part is missing or greatly reduced) in human beings.
* In chrysanthemum varieties like 'Kasturba Gandhi' from 'Mahatama Gandhi', 'Sonar Bangla' from 'Snow Ball', 'White Cloud' from 'Pink Cloud', 'Sharad Shobha' from 'Sharada' were delopped through spontaneous mutations.
* • In Bougainvillea, 'Jawahar Lal Nehru' is a bud sport mutant of cv. 'Lalbaugh' developed at IIHR, Banglore

**Advantages of Spontaneous Mutations**

There are several advantages of spontaneous mutations to organisms. For instance, it promotes diversification of traits within a population, mostly in physical features such as eye color. In bacteria, it ensures their survival in harsh conditions. For example, bacteria with a resistant trait against radiation or an antibiotic ensure their survival. Spontaneous mutation can raise the quality and nutritional value of plants used as food, e.g., sugary traits in maize are due to spontaneous mutation. Spontaneous mutation plays a role in forward genetics, where the bases pairs responsible for a particular physical trait are determined.

**IV.** **Induced mutagenesis**

A mutation known as an "induced mutation" is one that develops as a result of exposing an organism's DNA to a mutagen. The mutagen alters the chemical of the DNA, causing damage. The following are the causes of induced mutation:

Tautomers are compounds that share the same chemical formula but have different atom positions and atom bonds. They are the result of hydrogen protons moving about in DNA bases. Guanine, adenine, cytosine, and thymine are the bases (or nucleotides) of DNA. Physical agents, often known as mutagens, causes chromosome breakage Chemical mutagens: Mutations are caused by these substances' chemical composition and method of interaction with DNA bases. The mode of action of mutagens is quite different, and it may lead to: Addition or subtraction of base pairs Substitution of DNA bases Deletion of DNA bases Minor or more significant mutations can result from induced mutations. Micro lesions are another name for little mutations that only affect one base pair of the DNA. Large mutations, also known as macro lesions, are less frequent and are the opposite of small mutations. The nucleotides undergo significant modifications as a result. Point mutations are the most basic type of micro lesions. Point mutation happens when only one base of a DNA pair is altered. This entails a change in a gene at a particular location. A base pair in DNA can be added, deleted, or changed to cause a point mutation. Base substitution replaces one nucleotide with another, insertion includes the addition of a nucleotide, and deletion entails losing a piece of a DNA base pair. Induced mutations do not occur spontaneously. They are induced through various chemical and physical agents known as mutagens. Mutagens greatly enhance the frequency of mutation. Some of the mutagens are: Alkylating agents (Ethyl methanesulfonate or EMS, N-ethyl-N-nitrosourea or ENU) Base analogue (5-Bromouracil, Bromodeoxyuridine) Hydroxylamine modifies bases Deamination by nitrous acid DNA intercalating agents (ethidium bromide, proflavine) Oxidative damage (Reactive oxygen species, e.g. superoxide radical, hydrogen peroxide) Ionising and non-ionising radiations (gamma radiations, ultraviolet radiations, X-rays, etc.)

**Table 1: Difference between Spontaneous and Induced Mutation**

 The table below shows the main differences between Spontaneous and Induced Mutation.

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| --- | --- |
| **spontaneous Mutation** | **Induced Mutation** |
| Spontaneous mutations occur naturally and mainly due to error in replication | Induced mutations occur due to physical or chemical agents |
| Occurs due to slippage in natural processes | Induced by mutagens |
| Caused due to replication error, tautomeric shift, transposable genetic elements, unequal cross overs, etc. | Caused due to base modification, base analogues, intercalating agents, base mispairing, radiations, etc. |
| E.g. sickle cell anaemia | E.g. skin cancer due to prolonged exposure to radiations |

**Table 2: Basic classification of mutation on various basis.**

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| --- | --- |
| **Type of Mutation** | **Brief Description** |
| **1. Based on source** |
| Spontaneous | Mutation that occur in nature |
| Induced | Mutation which are produced by the use of mutagenic agent |
| **2. Based on direction** |
| Forward mutation | Any change from wild type allele |
| Reverse mutation | Any change from mutant allele to wild type |
| **3. Based on tissue** |  |
| Somatic mutation | A mutation in somatic tissue |
| Germinal mutation | A mutation in germ line cell |
| **4. Based on survival** |
| Lethal | A mutation which kills the individual that carries it. |
| Sub-lethal | When mortality is more than 50% of the individuals that carry mutation |
| Sub-vital | When mortality is less than 50% of the individuals that carry mutation |
| Vital | When all mutant individuals survive |
| **5. Based on site** |
| Nuclear mutation | A mutation in nuclear gene |
| Cytoplasm mutation | A mutation in cytoplasm gene |
| **6. Based on character** |
| Morphological | A mutation that alters the morphological characters of individuals |
| Biochemical | A mutation that alters biochemical function of individuals |
| **7. Based on visibility** |
| Micro-mutation | Mutation with invisible phenotypic changes. Generally observed in quantitative characters |
| Macro-mutation | Mutation with distinct morphological changes in phenotypes. Generally found in qualitativeCharacters |

**V. Mutagens**

**Table 3: physical mutagens**

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| --- | --- | --- | --- |
| **Mutagen** | **Source** | **Characteristics** | **Hazard** |
| X-rays | X-ray machine | Electromagnetic radiation; penetrates tissues from a few millimeters to many centimeters | Dangerous, penetrating |
| Gamma rays | Radioisotopes and nuclear reaction | Electromagnetic radiation produced by radioisotopes and nuclear reactors; very penetrating into tissues; sources are 60Co (Cobalt-60) and 137Cs (Caesium-137) | Dangerous, very penetrating |
| Neutrons | Nuclear reactors or accelerators | There are different types (fast, slow, thermal); produced in nuclear reactors; uncharged particles; penetrate tissues to many centimeters; source is 235U. | Very hazardous |
| Beta particles | Radioactive isotopes or accelerators | Produced in particle accelerators or from radioisotopes; are electrons; ionize; shallowly penetrating; sources include 32P and 14C | May be dangerous |
| Alpha particles | Radioisotopes |  Derived from radioisotopes; a helium nucleus capable of heavy ionization; very shallowly penetrating | Very dangerous |
| Protons | Nuclear reactors or accelerators | Produced in nuclear reactors and accelerators; derived from hydrogen nucleus; penetratetissues up to several centimeters | Very dangerous |
| Ion beam | Particle accelerators | Produced positively charged ions are accelerated at a high speed (around 20%–80% of the speed of light) deposit high energy on a target | Dangerous |

Source: Oladosu, Yusuff *et al*. (2015)

**VI. Chemical mutagenesis**

Mutation breeding initially relied heavily on physical mutagens. However, the discovery of substances with carcinogenic potential contributed to the continuously growing degree of understanding in mutation breeding. Chemical mutagens were originally described in detail by Auerbach and Robson in 1942. They claimed that in fruit flies, mustard gas can lead to chromosomal breakage and mutations. Since then, a number of these substances have been reported to have mutagenic potency comparable to that of physical mutagens. These substances include ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), ethylene imine (EI), diethyl sulphate (dES), N-nitroso-N-methylurea (NMU), N-nitroso-N-ethylurea (Sander and Muehlbauer, 1977). Chemical mutagens are simple to use, widely accessible, less expensive, more more precise and effective than physical mutagens (Khursheed *et al*., 2018). However, because chemical mutagens are potent carcinogens, sufficient precaution must be taken at every stage of mutagenic treatment. Research on crop mutagenesis has shown that chemical because mutagens have less severe effects on genetic components than ionizing radiation, which causes chromosome breakage, they are more favorable than radiation. According to Rapoport (1966), crop improvement efforts were using an enormously growing number of chemical mutagens. More than 390 mutant types have been created and formally released using chemical mutagens to far Rapoport has made a significant contribution by conceptualising the term "microgenetics," which provides information about gene structure and function, the mode of action of the mutagen and mutation, the origin of the mutation, and their fixation in the progeny. Rapoport uses chemical mutagenesis in his research. Sharma in 1985 presented a more thorough account of mutagens despite the fact that there are still many outstanding problems regarding their mechanism of action. Numerous mutagens are categorised as alkylating agents based on their ability to alkylate different genetic material locations as well as their potential to cause mutagenic action. Some of the often utilised alkylating agents include EMS, MMS, dES, NMU, and NEU. Replacement of the hydrogen in nitrogenous bases with the mutagen's alkyl group is referred to as to as alkylation Ashburner (1989) and Sharma and Chopra (1976) reported the following effects of alkylation.

**Table 4: chemical mutagens**

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| Mutagen group | Example | Mode of action |
| Alkyliting agents | 1-methyl-1-nitrosourea(MNU),1-ethyl-1-nitrosourea(ENU),Methylmethanesulphonate(MMS),ethyl methanesulphonate (EMS),dimethyl sulphate (DMS),diethyl sulphate (DES),1-methyl-2-nitro-1-nitrosoguanidine (MNNG), 1-ethyl-2-nitro-1-nitrosoguanidine (ENNG),N-dimethylnitrousamide(NDMA), N-diethylnitrous amide (NDEA). | React with bases and add methyl or ethyl groups and, depending on the affected atom, the alkylated base may then degrade to yield an a basic site, which is mutagenic and recombinogenic, or mispair to result in mutations upon DNA replication. |
| Azide | Sodium azide | Same as alkylating agents. |
| Hydroxylamine | Hydroxylamine | Same as alkylating agents. |
| Hydroxylamine | Hydroxylamine | Same as alkylating agents. |
| Antibiotics | Actinomycin D , Mitomysin C, Azaserine, streptonigrin etc. | Chromosomal aberrations also reported to cause cytoplasmic male sterility. |
| Nitrous acid | Nitrous acid | Acts through deamination, the replacement of cytosine by uracil, which can pair with adenine and thus through subsequent cycles of replication lead to transitions. |
| Acridines | Acridine orange | Intercalate between DNA bases thereby causing a distortion of the DNA double helix and the DNA polymerase in turn recognizes this stretch as an additional base and inserts an extra base opposite this stretched (intercalated) molecule. This results in frame shifts, i.e. an alteration of the reading frame. |
| Base analogues | 5-bromouracil (5-BU), maleic hydrazide,5-bromodeoxyuridine,2-aminopurine (2AP) | Incorporate into DNA in place of the normal bases during DNA replication thereby causing transitions (purine to purine or pyrimidine to pyrimidine); and tautomerization (existing in two forms which interconvert into each other, e.g. guanine can exist in keto or enol forms). |

**VII. Site‐directed mutagenesis**

Mutagenesis is frequently used to better understand the relationship between the structure and function of proteins and the regulatory regions of genes. Site-directed mutagenesis can be classified into two categories:

 Simple mutations and numerous mutations, depending on how many sites need to be altered. Methods for detecting single mutations rely on complementary oligonucleotides harboring the desired mutation to amplify double-stranded DNA from plasmids. This is one of the most popular methods for adding mutations to DNA fragments because of how easy it is, how little time it takes, and how effective it is. For multiple mutations, procedures either achieve the necessary mutations after several rounds of mutations or incorporate the intended mutations simultaneously in the same reaction. There are several commercial kits available for straightforward mutagenesis. Although these kits are simple to use, they frequently struggle to obtain substantial deletions. Additional techniques have been created for other applications in an effort to get over the restrictions of commercial kit.

Site-directed mutagenesis enzymes Versions of the high-fidelity DNA polymerases are often available for site mutagenesis in order to guarantee an accurate amplification by PCR. Low mistake rates are a typical characteristic of these polymerases. In order to delete incorrectly integrated nucleotides, high-fidelity DNA polymerases have a proofreading domain with polymerase activity 5' 3' and exonuclease activity 3' 5'. PfuTurbo and KOD, two DNA polymerases, are excellent tools for amplifying products. Phusion DNA polymerase and others cannot, however, when using comprehensive primers. The failure is probably caused by Phusion's need for high annealing temperatures, which are able to encourage the production of a duplex with perfectly matched complementary primers rather than one with mismatched primers and template. Utilizing a methylation-recognizing nuclease, such as DpnI, to destroy the template is a crucial component of site-directed mutagenesis. While fully methylated parental DNA can be removed by DpnI digestion, about 20–30% of hemimethylated molecules (parental strand mixed with PCR-generated strand) cannot be eliminated due to hemimethylated DNA, making the PCR product more resistant to DpnI.

**VIII. Procedure for mutation breeding**

Treating a biological material with a mutagen in order to induce mutations is known as mutagenesis. Exposure of a biological material to a radiation like X rays,\_gamma - rays, etc. is known as irradiation. When mutations are induced for crop improvement, the entire operation of the induction and isolation, etc. of mutants is termed as mutation breeding. A mutation breeding programme should be clearly planned and should be large enough with sufficient facilities to permit an effective screening of large populations. The various steps involved in mutation breeding are briefly discussed below.

**Objectives of the Programme**

 A mutation breeding programme should have well defined and clear-cut objectives. If the experimenter starts a mutagenesis programme just with the hope that he will discover something useful, he is most likely wasting his time and resources. This is because the ratio of beneficial to useless mutations is very small (1 in 800 mutations, that is, about 0.1 % of the mutations), and identifying desirable mutations from among the undesirable ones is a very difficult task indeed. Further, if a character governed by oligogenes is to be improved, the procedure for the handling of treated populations would be different from that when a polygenic trait is the target for improvement.

**Selection of the Variety for Mutagen Treatment**

Generally, the variety selected for mutagenesis should be the best variety available in the crop. This is particularly so when polygenic traits are to be improved. It serves no purpose to isolate desirable mutants in a less adapted inferior variety only to discover that the mutant lines have no agricultural worth, or that the mutants have to be used in a hybridization programme for transferring the mutant characteristics to a superior variety. In certain situations; however, it may be desirable to isolate mutants in varieties other than the best one. For example, an extensive search is being made for alternative dwarfing genes in cereals, particularly in wheat and rice (O. sativa). In this situation, dwarf and semi dwarf mutants would have to be isolated from tall varieties, which obviously would not be the best varieties of these crops.

**Plant part used for treatment**

Seeds, pollen grains or vegetative propagules (buds and cuttings) or even complete plants may be used for mutagenesis. Which plant part should be used for mutagen treatment depends primarily on whether the crop is sexually or asexually propagated and on the mutagen to be used. In sexually propagated crops, seeds are the most commonly used plant part. Dry dormant seeds are biologically almost inert and they can stand a range of extreme environmental conditions, such as, soaking, desiccation, heating, freezing, oxic or anoxic regimes, etc. Mutagenic treatment of seeds is essentially a treatment of embryo meristems.

Since mutation is a single cell event, the M1 plants will carry an induced mutation only in parts of the shoot, i.e., they will be chimaeras. Pollen grains may be used, but they are infrequently used because

* it is difficult to collect large quantities of pollen grains in most crop species,
* hand pollination (with treated pollen) is difficult, and
* Pollen al is relatively short. Pollen grains are the only plant part, which can be successfully treated with UV radiation.

A pollen monolayer is exposed to UV rays of 250 to 290 nm, of the biological effects induced by UV rays are almost comparable to those produced sparsely ionizing radiation. In case of clonal crops, buds or cuttings are used for mutagenesis. Radiation (except UV) is suitable for use with all the three plant parts and even with the plants. Whole plants are generally irradiated during the flowering stage so that it is equivalent to the irradiation of pollen grains and egg cells. However the treatment of whole requires special facilities (a gamma garden) and is possible in a few places only. Chemical mutagens are best used with seeds, but some workers have used them with vegetative propagules as well.

**Dose of the Mutagen**

The usefulness of a mutagen and the type of treatment required to obtain a high efficiency pendent upon specific properties of the mutagenic agent employed (its effectiveness, effect relationship and mode of application) as well as on specific characteristics of the biological system to be treated (the sensitivity of the treated tissues depending upon anatomical, physiological, biochemical and genetic peculiarities). The most appropriate plant or stage to be treated requires a thorough knowledge of the organisms and a clear definition of experimental objectives.

Mutagen treatments reduce germination, growth rate, vigour and fertility (pollen as well lie). There is considerable killing of plants during the various stages of development after mutagen treatment; thus survival is reduced considerably. Mutagens generally induce a high frequency of chromosomal changes and mitotic and meiotic irregularities. Usually, the damage increases with the mutagen dose, but it may not necessarily be proportional. An optimum dose is the one, which produces the maximum frequency of mutations and causes the minimum killing. The dose required for high mutagenic efficiency depends on the properties of the mutagenic agent, of the solvent medium and of the biological system. Many workers that a dose close to LD50 should be the optimum. LD50 is that dose of a mutagen, which would kill 50 per cent of the treated individuals. LD50 varies with the crop species and with mutagen used. A preliminary experiment is generally conducted to determine the suitable mutagen dose. In general, an overdose is likely to kill too many treated individuals, while an under dose would produce too few mutations. Dose of the mutagen may be varied by varying the intensity or the treatment duration. In case of radiation, intensity may be varied by changing the radiation source or by changing the nee from the radiation source of the material being irradiated. Intensity in the case of chemical mutagens may be varied by changing the concentration of mutagens.

**Giving Mutagen Treatment**

The selected plant part is exposed to the desired mutagen dose. In case of irradiation, the plant parts are immediately planted to raise M1 plants from them (pollen grains are used for pollination). In case of chemical mutagens, seeds are usually presoaked for a few hours to initiate metabolic activities, exposed to the desired mutagen and then washed in running tap water to remove the mutagen present in them. The treated seeds are, usually, immediately planted in the field to rise the M1 generation. M1 is the generation produced directly from the mutagen-treated plant parts without recourse to sexual or asexual reproduction. But when pollen grains are treated, the generation resulting from the seeds that were produced by pollination with the treated pollen grains would be the M1 generation. M2, M3, M4, etc. are the subsequent generations derived from M1, M2, M3, and etc. plants through selfing or clonal propagation.

**Handling of the Mutagen-Treated Population**

 Treatment of seeds and vegetative propagules commonly produces chimaeras. A chimera is an individual with one genotype in some of its parts and another genotype in the others. Shoottip meristem usually has three functional layers as follows:

(1) L1 gives rise to epidermis,

(2) L2 produces a part of leaf mesophyll and gametes, and

(3) L3 yields the rest of plant body.

When the whole of Ll, L2 or L3 layer is affected, the chimaera is known as periclimal chimaera, while in a sectorial chimaera only a part of Ll, L2 or L3 layer is affected. In sexually reproducing species, only the L2 chimaera (periclinal or sectorial) will be transmitted to the next generation; other chimaeras will not be recovered since these layers do not contribute to the production of gametes. In clonal crops, however, all chimaeras can be utilized either as periclinal chimaeras or by producing homogeneous individuals through sexual reproduction (only if the L2 layer is affected), tissue culture or certain other horticultural manipulations, e.g., wounding, etc., which induce production of adventitious shoot buds (all chimaeras are utilized). Sectorial chimaeras are unstable in clonal crops and have to be made periclinal through successive clonal propagation and selection for stability.

Mutations usually occur in small sectors of the meristem and, as a result, only a part of the plant is affected. One or more sexual or clonal generations coupled with selection are necessary to obtain a stable mutant phenotype. Mutant alleles are generally recessive, but some dominant mutations may also occur. In case of sexually reproducing crops, mutation breeding utilizes both recessive and dominant mutations and, in addition, excellent opportunities exist for mutation breeding for polygenic traits. Mutation breeding in clonal crops, however, primarily depends on dominant mutations; recessive mutations may also he utilized provided the clone used for mutagen treatment was heterozygous for the gene in question. For example, if recessive mutant allele a is to be useful in a clonal crop, the clone used for mutagenesis has to have the genotype Aa. Such situations are, however, rare; more frequently, the mutants useful in the improvement of clonal crops contain dominant mutations, and they may even include changes in chromosome structure or even number. Mutations are called macro or micro-mutations depending on the magnitude of phenotypic effect produced by them. A macromutation produces a large phenotypic effect recognizable on individual plant basis; obviously, such mutations are oligogenic in nature and can be easily selected in the M2 generation.

 In contrast, a micromutation has a small phenotypic effect that cannot be recognised on individual plant basis; it can be detected only in a group of plants and often statistical treatment of data may be necessary. Obviously, macromutations are polygenic in nature and selection for them is delayed till M3 or a later generation. The detailed procedure for handling of M2, M3, and etc. generations will differ depending mainly on the oligogenic or polygenic trait to be improved and on the mode of reproduction crop species.

The following discussion is based on sexually reproducing species, more particularly, self-pollinated species. Since dominant mutations are able to express themselves in heterozygous state, mutant plants are selected in M1 and often in M2 and M3, individual plant progenies are raised and homozygous mutants are selected. Selection for recessive mutations, however, can be taken up in M2 only, but the mutant allele will be homozygous in the M2 itself. Selection for polygenic traits is delayed till M3 generation, and is based on individual plant progenies rather than on individual plants. Generalized schemes handling the mutagen treated populations for oligogenic and polygenic traits are outlined in the next section.

**Table 5: procedure of mutation breeding**

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| Mutation breeding for polygenic traits |
| 1 year | M1 | Treated seeds are planted.Seeds from individual plant harvested separately. |
| 2year | M2 | Individual plant progenies are grown.Fertile vigorous and normal looking plants are harvested separately. |
| 3 year | M3 | Individual plant progenies of selected plant are grown.Superior plant selected from superior progenies showing segregation. |
| 4 year | M4 | Individual plant progenies of selected plant are grownSuperior and homogenous lines harvested in bulkSegregating lines usually rejected. |
| 5 year | M5 | Preliminary yield trial with suitable checkSuperior line selected. |
| 6-8 year | M6-M8 | Replicated yield trial at several location.Outstanding line released as new variety. |
| 9 year | M9 | Seed multiplication for distribution. |

**Handling of the mutated populations in the case of seed propagated species**

 All the treated seeds are grown to produce the M1 population. Generally the mutations will be recessive and most of them can be selected only in later generations. However, dominant mutations and pseudo-dominant mutations can be selected in the M1 itself. The M1 plants are selfed and the seeds are harvested separately. The M2 generation is raised from the seeds collected from the M1 generation. Oligogenic mutations can be selected at this level. Their seeds are grown separately and desirable mutants isolated after necessary trials. Superior and desirable M2 plants are selected and M3 seeds are collected. M3 progenies are raised from the seeds and they are evaluated for breeding behavior. The seeds of true breeding progenies are bulked together to conduct yield trials. Preliminary yield trials are conducted in the M4. Co-ordinated yield trials are carried out from M5 onwards. By M8 or M9, the most promising lines are selected and released. In the case of polygenic traits, inferior plants are rejected at M3 and M4 levels and based on screening tests, the remaining seeds are bulked and used for yield trials and finally released as new varieties.

**Handling of mutated populations in the case of clonally propagated species**

In vegetatively propagated species, mutations are expressed as chimeras. Chimeras are combinations of genetically different tissues. In the case of vegetatively propagated crops, the generation raised from the treated propagules is called the VM1 generation. Plants showing chimeras can be selected and propagated to produce the VM2 generation. Solid mutants are identified and selected in VM2. In VM3, the mutations identified in VM2 are confirmed. Preliminary yield trials are carried out in VM4 and co-ordinated trials from VM5 onwards. By VM9 the best line is released as a new variety.

**IX. Gamma gardens**

Gamma gardens are research facilities where crops are exposed to radiation in a controlled setting for a shorter time than the environment to test for mutations. After the awful atrocities of Hiroshima and Nagasaki, gamma gardens as these atomic gardens are often known were created as a way to find "peaceful" uses for atomic energy.

One strategy was to subject plants to nuclear radiation bombardment in an effort to cause several mutations, some of which, the researchers hoped, might be beneficial. For instance, it was hoped that these mutations would result in plants with bigger fruit or disease resistance. The tests were initially carried out by the top nuclear experts in the world in enormous atomic gardens on the grounds of national laboratories in the US and Europe. Atomic gardening involves exposing seedlings to various chemicals or blasting them with gamma rays, ion beams, and electrons to create random DNA changes. Most frequently, nuclear physicists would place seeds in dangerous cabinets or vast fields to expose them to radioactive cobalt-60. Around the radiation source in the middle of the field, scientists would often arrange the plants in circular split into wedges. The plants that received lower doses behind those that received higher doses typically thrived with new and occasionally fascinating features, as was expected, while the plants closest to the radiation source largely died. The radiation source would then be lowered back into the ground by technicians, who would subsequently scan the region for radioactivity. The plants that were successfully breeding desirable traits would subsequently be grown by scientists in protective gear, which would then disperse their seeds to unidentified facilities.

**X. Application of mutation breeding**

It has been applied to enhance physiological and morphological traits, disease resistance, and quantitative traits like yielding capacity.

* It helps to improve a few particular traits of a high-yielding variety that is well suited. This is especially helpful for floriculture crops, which are typically propagated through clones. These plants have a significant degree of heterozygosity. Therefore, in this situation, the only way to alter the genetic makeup of clones without modifying their unique traits is through mutagenesis.
* The induction of desired mutant alleles that might not otherwise be found in the regular population of germplasm or might exist but be unavailable to the breeder for ethical or practical reasons.
* It may also be applied to F1 hybrids or offspring produced by intervarietal crossings. These may be exposed to mutagens to promote recombination between related genes and increase genetic variety by causing mutations.
* Inter-specific (remote) hybrids have been exposed to radiation in order to create translocations.
* Mutations can result in an endless variety of variations. Mutation breeding frees plant breeders from total reliance on the natural world for basic resources.
* Mutation breeding is the sole option when progress is impossible, regardless of whether a variation is present or not via normal approaches.
* Mutation breeding is done to improve crops as much as possible.
* Mutation breeding is used once all naturally occurring diversity has been accounted for.

**XI. Limitations of mutation breeding**

Mutation breeding is the use of induced mutations for crop development. The following are the primary drawbacks of mutant breeding for crop development: For polyploidy species to breed mutations, high dosages of mutagens are required, and this process is frequently challenging. Mutations frequently result in pleiotropic effects. Desired mutants typically don't happen very often. They might have an impact on the crop variant's viability. Mutation breeding is an ineffective technique for crop development due to the lack of comprehensive information on mutagens. Lethal mutations are those that can occasionally be fatal to the organism. For the purpose of choosing the variants with the required mutations, a large population of crops must be screened. This procedure is frequently time-consuming and difficult. Certain crops with planned mutations could display unwanted side effects. To choose the variations with the necessary mutations, a vast population of crop species must be screened. This procedure is frequently time-consuming and difficult. Some crops that have undergone the desired mutation may experience negative side effects. Due to the dominance of its allelic counterpart, the majority of mutations are recessive, making it challenging to identify clonal crops that have recessive mutations. It might be difficult to register the mutant variety for commercial use in many places.

**XII. Achievements in some important field crops**

Rice and Wheat has been a special focus of mutation breeders. Over the last fifty years some 800 improved varieties of rice have been released globally either directly through mutation or by crossing these mutants with other breeding lines. KT 20-74 and SH 30-21 are the first rice varieties to be developed and released in China in 1957. Then Yenhsing-1 was developed through cross breeding programme with a mutant. Zhefu 802, a gamma irradiated rice mutant besides being a high yielding even in the poor conditions is resistant to rice blast also. It was the most widely planted rice variety between 1986-1994 in Republic of China. Japan released a semi dwarf rice variety “Reimi” that is a potential lodging resistant and high yielding mutant.

In India, several high yielding mutant varieties of rice were released under the series of PNR by IARI,New Delhi during 1970s and 1980s.These mutants being high in yield are short and early in maturity .This series provides us the PNR-381 and PNR-162 that are two aromatic and early ripening varieties of rice popularly cultivated in Haryana and Uttar Pradesh. Similarly gamma irradiated varieties of rice RD-6 and RD-15 released in Thailand in 1977.RD-6 which is an aromatic variety has a valuable glutinous endosperm and RD-15 is an early ripening variety. PL-12A Thermo sensitive Genic Male Sterile (TGMS) mutants of Japonica rice is controlled by a single recessive gene has a huge contribution for the production of hybrid rice varieties these outstanding mutants of soybean that are resistant to diseases, cold as well as tolerant to heat are cultivated there to harvest three crops in a single year [28]. In 2014 Vietnam produced 17 mutant varieties in rice, 10 in soybean, 2 in maize and 1 in chrysanthemum, that are officially and successfully released to Vietnam farmers. 50% of soybean and 15% of rice are produced from mutant varieties only. Gines is a rice mutant created by proton irradiation in Cuba, which thrives good in salty conditions, Giza-176 and Sakha-10 are high yielding rice mutants produced in Egypt, and further irradiating IR-5 rice in Myanmar gave a mutant “Shwewartun” which matures early with better yield and good grain yield.

Sharbati Sonara wheat having amber grain colour with early maturity and high yield protein content was developed through 200 Gy gamma irradiation in India. It was officially approved in 1967 and released to the farmers for cultivation. This wheat variety played a major role in green revolution in India. Durum wheat, which is a creso mutant, was especially created with thermal neutrons in Italy. Golden Promise barley is a gamma irradiated semi dwarf & salt tolerant mutant released in United Kingdom , which is used to make whisky and beer. Luther and Pennrad barley are two high yielding mutant varieties in USA.

Source: Mutation Breeding. [www.wikipedia.org](http://www.wikipedia.org)

 Mung Bean Mutants- Co-4, plant Mung-2 and TAP mung bean mutants.

Cotton Mutants - MA-9 Cotton. It is world’s first mutant in cotton that is drought tolerant and high yielding developed through X-ray irradiation and released for cultivation in 1948. Black Gram Mutants - MUM-2, LGG-407, LGG-450, CO-4, Dhauli (TT9E) & Pant-moong-1. They are virus resistant varieties of black gram and Tau-1.

Chick Pea Mutants- Pusa-408 (Ajay), Pusa-413 (Atul), Pusa-417 (Girnar) & Pusa-547. Ground Nut Mutant- TG-24 and TG-37

**Table 6: Brief description of some mutants**.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trait category** | **Mutant’s name (*species*)** | **Description** | **Development type** | **References** |
| Agronomic and botanic traits | ‘Above’ (*Triticum aestivum* L.) | Awned, white glumed, early matur- ing and semidwarf | Treatment of seed with chemical mutagen, sodiumazide (NaN3) | Newhoue et al. ([1992](#_bookmark41)) |
| Quality and nutrition traits | ‘Aldamla’(*Prunus avium* L.) | Compact growth habit (70–80 %), longpetioles and improved fruit quality | Irradiation of dor- mant buds with gamma rays | Kunteret al. ([2012](#_bookmark30)) |
| Resistance to biotic stresses | ‘Akita Berry’ (*Fragaria x ananassa*) | Improved resistance to black leaf spot dis- ease (*Alternaria**alternata*) | Somaclonal muta- tion by meristem culture | MVD ([2016](#_bookmark39)) |
| Tolerance to abiotic stresses | ‘Maybel’ (*Lycopersicon esculentum*M.) | Very high perfor- mance under drought conditions | Treatment of seed with gamma rays | MVD ([2016](#_bookmark39)) |
| Yield and contributors | ‘Early Blen- heim’ (*Prunus armeniaca* L.) | Early maturity, higher yield, large fruits and self-compatible pollen | Treatment of dor- mant scions with thermal neutrons (thN) | Sigurbjoernsson and Micke ([1974](#_bookmark49)) |

**Achievements in some important ornamental crops**

Several varieties have also been evolved through natural mutations or as bud sports of existing varieties. At IARI, 3 rose varieties were developed through induced mutations are 'Abhisarika', 'Pusa Christina' from 'Christian Dior', and 'Madhosh’.

**Carnation:**

IIHR, Bangalore has released the first variety in India as Arka Flame as a result of in vitro mutation breeding. Recently another variety Arka Tejas has been released. At I.A.R.I., New Delhi, experiments on mutation breeding were carried out. Seeds of different lines of carnation have been irradiated with 6 to 20 kr. dosage of gamma rays and some interesting mutants with variegated leaf were obtained (Kaicker, 1988).

**Zinnia**:

By recurrent selection from the irradiated seeds of *Zinnia elegans* a mixed coloured variety resistant to leaf curl virus has been evolved at IARI, New Delhi (Swarup and Raghava, 1974).

**Rose:**

* Pusa Christiana: Mutant of 'Christian Dior, gamma rays
* induced Abhisarika: Mutant of 'Kiss of Fire
* Madhosh: Mutant of Gulzar, EMS (i.e. 0.025% for 8 hours)
* Angara: Mutant of Montezumma
* Sharda: Mutant of Queen Elizabeth

**Bougainvillea:**

* Los Banos Variegata: Gamma-ray induced mutant of multibracted bougainvillea cultivar Los Banos Beauty
* Los Banos Variegata Silver Margin: Gamma-ray induced mutant of multibracted bougainvillea cultivar Los Banos Beauty
* Mahara Variegata: Gamma-ray induced mutant of multibracted bougainvillea cultivar Mahara.
* Bougainvillea: Los Banos Variegata Jayanthi: Ethyl Methane Sulphonate (EMS) induced chlorophyll variegated mutant of bougainvillea cv. Los Banos Beauty.

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