Mutation Breeding in Crop Improvement

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Abstract

Plant breeding relies on genetic variation as its main tool to create unique and improved cultivars. Fortunately, mutant breeding offers us hope for the development of food crops with high nutritional quality yields that also increase the content and bioavailability of vital elements. Additionally, they create crop kinds that can withstand salt, drought, and disease. The role of mutagenic plants and their role in human food systems are less understood, despite the fact that the role and abundance of variations of transgenic crops in human food systems and their effect on ecology, human health, and agriculture biodiversity are better understood and well documented.

Since the X-ray effects on mutation were discovered, mutation breeding has emerged as a promising and unmatched method for the improvement of crops. Even if new and cutting-edge methods for inducing mutations have emerged, they have always aided in the fundamental goal of improving crops to meet population growth demands for food security, sustainable nutrition, and improved nutrition. The general reduction in genetic diversity that has been steadily occurring in crop species is also partially offset by the heritable variability brought on by the mutations.

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. . Since Stadler first published studies on the mutations generated by irradiation in maize and barley, radiation has been widely exploited to create novel cultivars used for crop production and as genetic resources. Due of its wide mutation spectrum and high mutation effectiveness, radiation mutation breeding has unparalleled advantages over other breeding methods, such as cross-breeding and chemical mutagenesis. 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 on 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 hypotheses, the genesis of plant mutation can be pinpointed to tales of mutant crops in China around 300 BC. In 1791, an English farmer named Seth Wright noted the first instance of a mutation in a male lamb with an unusually short leg. Hugo de Vries first applied the term "mutation" in 1900 after observing it in the plant *Oenothera lamarkiana*. The genetic analysis of the drosophila white eye mutant by Morgen in 1910 marked the beginning of the systematic study of mutation. By employing X-rays, Muller 1927 causes mutation in drosophila. In 1946, he received a noble prize. Hugo de Vries was the first to identify mutations as a mechanism for creating variation in the late nineteenth century while working on the "rediscovery" of Mendel's principles of heredity.

According to him, the mechanisms underlying this variety are fundamentally distinct from those underlying segregation and recombination. He described this phenomena as abrupt, hereditary changes in organisms that significantly affect how they seem phenotypically. He defined this phenomenon as sudden alterations in organisms that were hereditary and had a large impact on the phenotypic characteristics of the organisms. The study of radiation-induced mutations as a method for generating distinctive genetic variation in plants expanded when Stadler identified the mutagenic activity of X-rays in maize, barley, and wheat.

The first strain of commercial mutant tobacco was developed in 1934. 77 cultivars were produced using mutagenesis before to 1995, according to Acquaah. In 1995, there were 484 types that were offered for sale. This number has dramatically increased since then. Only a few examples of the plants include fruit trees (banana, mango, papaya, etc.), ornamentals (rose, gerbera, marigold, etc.), and food crops (chicken pea, wheat, green gramme, etc.). Mutant breeding has changed agronomic traits such lodging resistance, early maturation, winter hardiness, and product quality.

Why are we interested in Mutants?

Mutation is the primary cause of variation. Some mutations result in newer protein versions and aid organisms in adjusting to environmental changes. Because it modifies the DNA sequence of a particular gene, the mutation, which is the first stage of evolution, creates a new allele. Through iatrogenic recombination, a novel DNA sequence could be produced for a particular gene.

III. SPONTANEOUS MUTATION

According to science, spontaneous mutations result from the accumulation of all possible DNA errors that could occur during an organism's life cycle (Glickman et al., 1986). Therefore, the types and amounts of spontaneous mutations are produced by the combination of all mutagenic and anti-mutagenic biological systems. It is not well known that fresh modifications in experimental settings greatly modify the kinds and rates of spontaneous mutations. Base substitutions, frame shifts, insertions, and deletions are all examples of mutations that happen spontaneously. However, the literature has paid very little attention to the study of the mechanisms behind spontaneous mutagenesis and the minor experimental variables that affect the types and frequencies of spontaneous mutations. This is unfortunate because it appears that spontaneous mutagenesis plays a significant role in cancer, ageing, and evolution. This review focuses on small experimental parameters that have a big impact on the results of a spontaneous mutation experiment. Once these factors are sufficiently understood, a hypothesis of "directed" mutagenesis is not required. We look at the genetic control over spontaneous mutagenesis, DNA's inherent instability, and the different kinds of normal metabolic lesions that develop in DNA and cause mutations through errors in replication, repair, and recombination. Similar to spontaneous mutagenesis, spontaneous carcinogenesis is seen as the accumulation of all potential DNA blunders that may happen throughout the lifetime of an organism. Spontaneous mutations occur naturally in the genome. Typically, they originate from an error during replication, mitosis, meiosis, or another process. Mutations can also be brought on by mobile genomic elements called transposons.

Typically, spontaneous mutations occur more frequently one in 10 lacs, i.e., 10^{-6}.

eg. shrunken seeds, purple color, colorless or sugary traits in corn or maize plant, Lactose fermentation character in Escherichia coli, Radiation resistance trait in Escherichia coli, etc

Advantages of Spontaneous Mutations

Spontaneous mutations have several benefits for organisms. For example, it encourages the diversification of traits among a population, mostly in terms of physical characteristics like eye colour. It guarantees bacteria's survival in challenging circumstances. For instance, germs

that are resistant to radiation or an antibiotic are more likely to survive. The quality and nutritional content of plants used as food can be improved by spontaneous mutation; for instance, sweet features in maize are the result of spontaneous mutation. Forward genetics, which identifies the base pairs in charge of a specific physical attribute, makes use of spontaneous mutation.

IV. INDUCED MUTAGENESIS

When an organism's DNA is exposed to a mutagen, a mutation known as a "induced mutation" results. The mutagen damages DNA by changing its chemical structure. The reasons of induced mutation include the following:

Tautomers are substances with the same chemical structure but distinct atom arrangements and atom bonds. They arise from the motion of hydrogen protons within DNA bases. The four bases (or nucleotides) that make up DNA are guanine, adenine, cytosine, and thymine. Chromosome breakage is caused by physical agents, also referred to as mutagens. Chemical mutagens: Due to the chemical makeup of these compounds and the way they interact with DNA bases, they can cause mutations. Mutagens work in a very distinct way, and their effects can include the following: Base pair addition and subtraction replacement of DNA bases base deletion in DNA Induced mutations might lead to less significant or more serious mutations. Small mutations that only alter one base pair of the DNA are known as micro lesions. The opposite of minor mutations are large mutations, usually referred to as macro lesions, which are less common. As a result, the nucleotides experience substantial changes. The most fundamental kind of micro lesions are point mutations. When one base in a DNA pair is changed, this is referred to as a point mutation. A certain gene must change in order for this to occur. Point mutations in DNA can result from the addition, deletion, or modification of a base pair. Base substitution involves switching out one nucleotide for another, while insertion involves a nucleotide is added when a base pair in DNA is lost when a nucleotide is deleted. Mutations that are induced do not happen naturally. They are brought about by a variety of physical and chemical substances known as mutagens. Mutation frequency is significantly increased by mutagens. Several of the mutagens include: Alkylating substances (Ethyl methanesulfonate or EMS, N-ethyl-N-nitrosourea or ENU) baseline analogue (5-Bromouracil, Bromodeoxyuridine) Bases are modified by oxylamine. Nitrous acid DNA intercalating agents deaminating (ethidium bromide, proflavine) Oxidative injury (Reactive oxygen species, e.g. superoxide radical, hydrogen peroxide) Radiation both ionising and non-ionizing (gamma radiations, ultraviolet radiations, X-rays, etc.)

Table 1: Difference between Spontaneous and Induced Mutation

The key distinctions between spontaneous and induced mutation are shown in the table below.

Spontaneous Mutation	Induced Mutation
Naturally occurring spontaneous mutations are mostly caused by replication errors.	Aspects such as physical or chemical factors might cause induced mutations.
It is due to slippage in natural processes	Induced by mutagens
Caused due to transposable genetic elements, unequal cross overs, tautomeric shift etc.	Caused due to base analogues, intercalating agents, base modification, base mispairing, radiations, etc.
Eg. sickle cell anaemia	Eg. Skin cancer brought induced by extended radiation exposure

Table 2: Basic classification of mutation on various basis.

Type of Mutation	Brief Description
1. According to source	
Spontaneous	Unplanned mutations that happen naturally.
Induced	Mutations brought about by the usage of mutagenic substances.
2. According to direction	
Forward mutation	Alterations from the wild type allele.
Reverse mutation	Changing a mutant allele to a wild type in any way.
3. According to tissue	
Somatic mutagenesis	Somatic tissue mutation.
Germinal mutagenesis	Change in the germ line cell
4. According to survival	
Lethal	Mutation that is fatal to the person who bears it
Sub-lethal	When the mutation-carrying individuals' mortality rate exceeds 50%.
Sub-vital	When the mutation-carrying population is less than 50% deadly
Vital	When every mutant person lives
5. According to location	
Nuclear mutation	Nuclear gene mutation
Cytoplasm mutation	A mutation in cytoplasm gene
6. According to character	
Morphological	A mutation that modifies morphological characters of species.
Biochemical	Biochemical a mutation that modifies people's metabolic functions
7. According to visibility	
Micro-mutation	Invisible phenotypic changes brought on by mutation. Generally observed in quantitative characters
Macro-mutation	Mutation with distinct morphological changes in phenotypes. Generally found in qualitative characters

V. MUTAGENS

Table 3: physical mutagens

Mutagen type	Source	features
Gamma ray	nuclear reaction and Radioisotopes	Electromagnetic radiation availed by nuclear reactors and radioisotopes. sources are Cobalt-60 and Caesium-137
Beta particle	Radioactive isotopes or accelerators	Produced in particle accelerators or from radioisotopes, are electrons; ionize, low penetrating, sources include ^{32}P and ^{14}C
X-rays	X-ray machine	Electromagnetic radiation, penetrates into tissues from a few millimeters to many centimeters

Mutagen type	Source	features
Ion beam	Particle accelerators	Produces positively charged ions are accelerated at a high speed (around 20%–80% of thespeed of light) deposit high energy on a target tissue.
Proton	accelerators or Nuclear reactors	Produced in nuclear reactors and accelerators, they can penetrate tissues up to a few centimetres and are formed from the hydrogen nucleus.
Alpha	Radioisotopes	Produced from radioisotopes, a helium nucleus capable of heavy ionization, very low penetrating
Fast Neutron	Nuclear reactors oraccelerators	There are various types (fast, slow, thermal), produced in nuclear reactors, unchargedparticles highly penetrating; source is ^{235}U.

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

VI. CHEMICAL MUTAGENESIS

Physical mutagens were initially heavily used in mutation breeding. However, the identification of chemicals with cancer-causing potential added to our ever-increasing level of knowledge regarding mutant breeding. Auerbach and Robson first provided a thorough description of chemical mutagens in 1942. They asserted that mustard gas can cause chromosomal breaks and mutations in fruit flies. Since then, it has been claimed that a number of these chemicals have mutagenic potential on par with physical mutagens. These include N-nitroso-N-methylurea (NMU), N-nitroso-N-ethylurea, ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), ethylene imine (EI), diethyl sulphate (dES), and n-nitroso-N-ethylurea (Sander and Muehlbauer, 1977). Compared to physical mutagens, chemical mutagens are easier to utilise, more accessible, less expensive, more precise, and more effective (Khursheed et al., 2018). However, adequate precautions must be taken at every stage of mutagenic treatment because chemical mutagens are strong carcinogens. Research on crop mutagenesis has demonstrated that chemical mutagens are preferable to radiation because they have less detrimental effects on genetic elements. Ionizing radiation induces chromosome breakage. Rapoport (1966) claimed that an immensely rising number of chemical mutagens were being used in crop development attempts. Chemical mutagens have so far been used to manufacture and formally release more than 390 different mutant kinds. Rapoport made a crucial contribution by coining the term "microgenetics," which offers details on gene structure and function, the way mutagens and mutations work, where mutations come from, and how they are fixed in offspring. Rapoport's research on chemical mutagenesis Sharma gave a more complete explanation of mutagens in 1985, despite the fact that there are still many open questions about how they work. Based on their capacity to alkylate various genetic material loci as well as their potential to have mutagenic effects, a variety of mutagens are classified as alkylating agents. EMS, MMS, dES, NMU, and NEU are a few of the frequently used alkylating agents. Alkylation is the process of substituting the mutagen's alkyl group for the hydrogen atom in nitrogenous bases. According to Sharma and Chopra (1976) and Ashburner (1989), alkylation has the following effects.

Table 4: chemical mutagens

Mutagen groups	Example	Mode of action
Alkyliting agents	1-methyl-1-nitrosourea, 1-ethyl-1-nitrosourea, Methylmethanesulphonate, ethyl methanesulphonate , dimethyl sulphate , diethyl sulphate , 1-methyl-2-nitro-1-nitrosoguanidine , 1-ethyl-2-nitro-1-nitrosoguanidine, N-dimethylnitrousamide, N-diethylnitrous amide.	When bases and methyl or ethyl groups react, the alkylated base may either degrade to produce a basic site, which is mutagenic and recombinogenic, or mispair to cause mutations during DNA replication, depending on the affected atom.
Nitrous acid	Nitrous acid	Acts through deamination, the substitution of uracil for cytosine, can pair with adenine and lead to transitions during successive replication cycles.
Hydroxylamine	Hydroxylamine	Similar like alkylating agents.
Base analogues	5-bromouracil, maleic hydrazide, 5-bromodeoxyuridine, 2-aminopurine	Incorporation into DNA in place of the typical bases during DNA replication to cause transitions (purine to purine or pyrimidine to pyrimidine) (existing in two forms which converted into each other.
Azide	Sodium azide	Similar like alkylating agents.
Antibiotics	streptonigrin, Mitomysin C, Azaserine, Actinomycin D etc.	Chromosomal abnormalities also found to cause cytoplasmic male sterility
Hydroxylamine	Hydroxylamine	Similar like alkylating agents.
Acridines	Acridine orange	The DNA polymerase then interprets this stretch as an additional base and inserts an additional base opposite this stretched (intercalated) molecule, causing a deformation of the DNA double helix. As a result, there are frame shifts, or changes to the reading frame.

VII. SITE-DIRECTED MUTAGENESIS

The link between the regulatory regions of genes and the structure and function of proteins is frequently studied using mutagenesis. Two types of site-directed mutagenesis can be distinguished:

Depending on how many sites must be altered, there can be a single mutation or multiple mutations. Complementary oligonucleotides carrying the desired mutation are used to amplify double-stranded DNA from plasmids in methods for finding single mutations. Due to how simple, quick, and efficient it is, this technique is one of the most widely used for

introducing mutations to DNA fragments. For numerous mutations, processes either include the desired alterations simultaneously in the same reaction or arrive at the necessary mutations after several rounds of mutation. There are numerous commercial kits readily available for simple mutagenesis.

Despite being easy to use, these kits frequently have trouble obtaining significant deletions. In an effort to get over the limitations of commercial kit, other ways have been developed for other uses.

Enzymes for site-directed mutagenesis For site mutagenesis, high-fidelity DNA polymerases are frequently available in order to ensure accurate PCR amplification. These polymerases are known for having low error rates. High-fidelity DNA polymerases have a proofreading domain with polymerase activity 5' 3' and exonuclease activity 3' 5' to remove improperly integrated nucleotides. Two DNA polymerases that are great for amplifying products are PfuTurbo and KOD. When utilising thorough primers, Phusion DNA polymerase and others cannot. The requirement for high annealing temperatures by Phusion, which can stimulate the synthesis of a duplex with perfectly matched complementary primers rather than one with mismatched primers and template, is likely what led to the failure. Site-directed mutagenesis relies heavily on the destruction of the template using a methylation-recognizing nuclease, such as DpnI. Approximately 20–30% of hemimethylated molecules (parental strand combined with PCR-generated strand) cannot be removed due to hemimethylated DNA, making the PCR result more resistant to DpnI digestion while completely methylated parental DNA can be removed.

VIII. PROCEDURE FOR MUTATION BREEDING

Mutagenesis is the process of exposing biological material to a mutagen in order to cause mutations. Irradiation is the process of subjecting a biological material to radiation such as X rays, gamma rays, etc. Mutation breeding refers to the complete process of inducing, isolating, etc., mutants for the purpose of crop improvement. A mutation breeding programme should have a defined plan and be big enough and equipped with adequate resources to allow for efficient screening of huge populations. The next section includes a quick discussion of the various mutation breeding steps.

Selection of the Variety for Mutagen Treatment

Generally speaking, the crop's finest variety should be the one chosen for mutagenesis. This is especially true when improving polygenic features. It is useless to find desirable mutants in inferior varieties that are less adapted to their environment only to find that the mutant lines have no agricultural use or that the mutants must be employed in a hybridization effort to transfer the mutant traits to superior varieties. It might, however, be advantageous in some circumstances to isolate mutants from inferior kinds. For instance, a thorough search for alternative dwarfing genes in cereals, especially in wheat and rice, is being conducted (O. sativa). In this case, it would be necessary to separate dwarf and semi-dwarf mutants from obviously, the best variety of these crops would not be the tall varieties.

Plant part used for treatment

Mutagenesis can be carried out on seeds, pollen grains, vegetative propagules (buds and cuttings), or even whole plants. Whether a crop is propagated sexually or asexually and the

mutagen to be employed are the main factors in determining which plant component should be used for mutagen therapy. The most often used plant portion in crops that are sexually reproduced is the seed. Dry dormant seeds may withstand a variety of harsh climatic conditions, including soaking, desiccation, heating, freezing, oxic or anoxic regimes, etc. They are practically inactive physiologically. In essence, treating seeds with mutagenic agents means treating embryo meristems.

Because mutation occurs in a single cell, the M_1 plants will only have an induced mutation in some regions of the shoot, making them chimaeras. Pollen grains can be utilized, but they aren't often since it's hard to gather a lot of them from most crop species, it's challenging to hand pollinate with treated pollen, and pollen grains aren't very long. The only component of a plant that can be successfully treated with UV light is pollen grains.

The biological effects of UV rays, which are produced by wavelengths of light between 250 and 290 nm, on a pollen monolayer are virtually as strong as those of infrequently produced ionizing radiation. For mutagenesis in clonal crops, buds or cuttings are utilized. All three plant sections and even the plants can be exposed to radiation (apart from UV). During the blossoming period, whole plants are typically exposed to radiation so that it is comparable to the radioactive treatment given to pollen grains and egg cells. However, treating the whole person necessitates specialized equipment (a gamma garden) and is only feasible in a few locations. Although some researchers have utilized vegetative propagules as well, chemical mutagens are more effective when used with seeds.

Dose of the Mutagen

The usefulness of a mutagen and the type of treatment necessary to achieve a high efficiency depend on specific characteristics of the biological system to be treated as well as on the employed mutagenic agent's effectiveness, effect relationship, and mode of application (the sensitivity of the treated tissues depending upon anatomical, physiological, biochemical and genetic peculiarities). It is important to have a solid understanding of the organisms and clearly defined experimental goals in order to choose the best plant or stage to treat.

Mutagen treatments decrease fertility, vigour, growth rate, and germination (pollen as well lie). Following a mutagen treatment, there is a significant amount of plant death at various phases of development, which significantly lowers survival. Chromosome alterations are frequently brought on by mutagens. The damage often rises with the mutagen dose, though this is not always the case. An ideal dose is one that results in the greatest frequency of mutations while causing the least amount of mortality. The features of the mutagenic agent, the solvent media, and the biological system all have a role in determining the dose necessary for high mutagenic efficiency. Many employees believe that the best dose is one that is close to the LD50. LD50 is the amount of a mutagen that would cause 50% of those who were exposed to it to die. The LD50 varies depending on the type of crop and the mutagen used. To establish the ideal mutagen dose, a preliminary experiment is typically carried out. An overdose typically results in too many treated people dying, whereas an under dose would produce too few mutations. The strength or length of the treatment can change the mutagen dose. Changing the radiation source or the distance of the substance being irradiated from the radiation source can change the intensity of radiation. Chemical mutagens allow for the modification of intensity by altering mutagen concentration.

Mutagen Treatment

The desired mutagen dose is administered to the chosen plant section. In the event of irradiation, the plant pieces are immediately planted in order to produce M_1 plants (pollen grains are used for pollination). With the case of chemical mutagens, seeds are often presoaked for a few hours to kick-start metabolic processes, exposed to the desired mutagen, and then washed in running tap water to eliminate the mutagen present in them. In order to raise the M_1 generation, the treated seeds are typically planted right away in the field. The M_1 generation is that which is created solely by sexual or asexual reproduction from the mutagen-treated plant components. Nevertheless, if pollen grains are treated, the generation that emerges from the seeds formed by pollination with the treated pollen grains would be the generation M_1. The future generations, M_2, M_3, M_4, etc., are formed from the plants of generations M_1, M_2, M_3, and so on through selfing or clonal propagation.

Usually, only a small portion of the plant is impacted by mutations since they take place in discrete areas of the meristem. In addition to selection, one or more sexual or clonal generations are required to produce a stable mutant phenotype. Although most mutant alleles are recessive, occasional dominant mutations can also happen. Recessive and dominant mutations are both used in the case of sexually reproducing crops, and significant chances for mutation breeding for polygenic traits also exist. However, dominant mutations are primarily responsible for mutation breeding in clonal crops; recessive mutations can also be employed if the clone that underwent mutagen treatment was heterozygous for the relevant gene. For instance, if a clonal crop needs to use recessive mutant allele a, the clone used for mutagenesis has to have the genotype Aa.

These circumstances, however, are uncommon; more often than not, the mutants helpful in enhancing clonal crops involve dominant mutations, and they may even include alterations in chromosome structure or even number. Depending on the size of the phenotypic effect they cause, mutations are referred to as macro or micro mutations. A macro mutation has a significant phenotypic impact that can be seen in each individual plant; it is clear that these mutations are oligogenic in nature and are simple to select in the M_2 generation.

The explanation that follows is centered on species that can reproduce sexually, and more specifically, self-pollinated species. Since dominant mutations can manifest in the heterozygous state, mutant plants are generally picked in M_2 and M_3 as well as in M_1, and homozygous mutants are raised and selected from individual plant progenies. However, only the M_2 can be used for selection for recessive mutations, and even then, the mutant allele will be homozygous in the M_2. Till the M_3 generation, selection for polygenic traits is postponed, and it is dependent on individual plant progenies rather than individual plants. The next section describes generalized strategies for addressing oligogenic and polygenic features in populations that have been exposed to mutagens.

Table 5: procedure of mutation breeding

Mutation breeding for polygenic traits		
1 year	M_1	Treated seeds are sown. Seeds from single plant harvested individually.
2year	M_2	Single plant progenies are grown. vigorous and normal looking fertile plants are harvested separately.
3 year	M_3	Single plant progenies of noted plant are grown. Superior plant selected from progenies showing segregation.

Mutation breeding for polygenic traits		
4 year	M_4	single plant progenies of noted plant are grown Superior and homogenous lines harvested in bulk Segregating lines usually rejected.
5 year	M_5	Preliminary yield trial with best check best line selected.
6-8 year	M_6-M_8	Replicated yield trial at many sites. Superior line released as new variety.
9 year	M_9	Seed multiplication for distributed to farmers.

In the case of seed-propagated species, handling the altered populations

The M_1 population is created by growing all of the treated seeds. Most mutations are usually recessive and can only be selected in subsequent generations. However, the M_1 itself can be chosen for dominant mutations and pseudo-dominant mutations. Self-pollinated M_1 plants are harvested for their seeds separately. The seeds saved from the M_1 generation are used to rise the M_2 generation. At this level, oligogenic mutations are selectable. Their seeds are cultivated separately, and after the required testing, the desired mutants are isolated. The best and most appealing M_2 plants are chosen, and M_3 seeds are gathered. From the seeds, M_3 offspring are reared, and their breeding behavior is assessed. To conduct yield trials, the seeds of real breeding progenies are bulked together. Initial yield tests are carried out in the M_4. From M_5 onward, coordinated yield trials are conducted. The most promising lines are chosen and released by M_8 or M_9. When polygenic characteristics are present, poorer plants are discarded at the M_3 and M_4 levels. The surviving seeds are then bulked and utilized for yield trials before being released as new varieties.

Regarding clonally propagated species, how to handle mutant populations

In organisms that reproduce vegetatively, mutations manifest as chimaeras. Chimeras are blends of tissues with various genetic compositions. The generation that was raised from the treated propagules in the case of vegetatively propagated crops is known as the VM_1 generation. To create the VM_2 generation, it is possible to choose and propagate plants that exhibit chimaeras. In VM_2, solid mutants are found and chosen. The alterations found in VM_2 are verified in VM_3. Initial yield trials are conducted in VM_4, and coordinated trials start in VM_5. The greatest line is made available as a new variety by VM_9.

IX. GAMMA GARDEN

In order to screen for mutations, crops are exposed to radiation in gamma gardens, which are research facilities, for a shorter period of time than the environment would allow. Gamma gardens, as these atomic gardens are often known, were developed as a means to find "peaceful" uses for atomic energy following the terrible atrocities of Hiroshima and Nagasaki.

One tactic involved bombarding plants with radioactive radiation in an effort to mutate them in various ways, some of which the researchers hoped may be advantageous. For example, it was hoped that these mutations might produce plants that bore larger fruits or were resistant to disease. On the grounds of national laboratories in the beginning, the tests were conducted by the top nuclear scientists in the world in massive atomic gardens. Atomic gardening includes bombarding seedlings with gamma rays, ion beams, and electrons in order to induce

random DNA mutations in the plants. Nuclear physicists would typically expose seedlings to radioactive cobalt-60 by putting them in hazardous cabinets or large fields. Scientists frequently arranged the plants in a circle with wedges around the radiation source in the middle of the field. It was believed that the plants furthest from the radiation source would generally perish, whilst those that received lower doses behind those that received greater doses would typically thrive with new, occasionally fascinating traits. Technicians would then lower the radiation source back into the ground, after which they would scan the area for radioactivity. Scientists in protective gear would grow the plants that were successfully reproducing the desired features, and they would then distribute the seeds to undisclosed facilities.

X. APPLICATION OF MUTATION BREEDING

It has been used to improve physiological, morphological, and quantitative features including yielding capacity as well as disease resistance.

- A high-yielding variety that is well adapted can benefit from having a few specific features improved. This is particularly beneficial for crops used in floriculture, which are often propagated using clones. There is a sizable amount of heterozygosity in these plants. Therefore, under this circumstance, mutagenesis is the sole method available for changing the genetic make-up of clones without changing their distinctive features.
- The creation of desired mutant alleles that would not typically be present in the usual population of germplasm or that might already exist but are not accessible to the breeder for moral or practical reasons.
- It can also be used to describe F_1 hybrids or the progeny of inter varietal crosses.
- Translocations have been produced by radiation-exposed inter-specific (remote) hybrids; mutations can produce an infinite number of variants. Plant breeders can no longer completely rely on the natural world for their basic needs thanks to mutation breeding.
- Whether a variant is present or not through conventional methods, mutation breeding is the only choice when progress is unattainable.
- Mutation breeding is used to maximize crop improvement.
- Once all naturally occurring diversity has been taken into consideration, mutation breeding is used.

XI. LIMITATIONS OF MUTATION BREEDING

Utilizing induced mutations for crop development is known as mutation breeding. The main limitations of mutant breeding for crop improvement are as follows: High doses of mutagens are necessary for polyploidy species to breed mutations, and this procedure is sometimes difficult. Oftentimes, mutations have pleiotropic consequences. Rarely do desired mutants appear in nature. They might affect the viability of the crop variation. Due to the dearth of complete knowledge on mutagens, mutation breeding is a poor method for crop improvement. Lethal mutations are ones that occasionally cause an organism's demise. A vast population of crops must be screened in order to choose the variants having the necessary mutations.

This process is frequently challenging and time-consuming. Planned mutations may have unintended impacts on some crops. There is a large population of crop species that needs to be screened in order to choose the variations with the required mutations. This process is

frequently challenging and time-consuming. There may be unfavorable side effects in some crops that have undergone the desired mutation. The majority of mutations are recessive because their allelic counterpart predominates, making it difficult to identify clonal crops with recessive mutations. Registration of the mutant variety for commercial usage may be challenging in many locations.

XII. ACHIEVEMENTS IN SOME IMPORTANT FIELD CROPS

During the 1970s and 1980s, IARI, New Delhi developed a number of high yielding mutant rice varieties under the series of PNR. These mutants are short and mature quickly due to their high output. The two aromatic, early-ripening rice varieties PNR-381 and PNR-162, which are widely grown in Haryana and Uttar Pradesh, are provided to us in this series. In 1977, Thailand released the RD-6 and RD-15 gamma-irradiated rice varietals. A valuable sticky endosperm is present in the fragrant variety RD-6, and the early-ripening variation RD-15. A single recessive gene controls the PL-12A Thermo Sensitive Genetic Male Sterile (TGMS) mutants of Japonica rice, which greatly contribute to the creation of hybrid rice varieties.

In 2014, Vietnam generated 17 mutant rice varieties, 10 mutant soybean varieties, 2 mutant maize varieties, and 1 mutant chrysanthemum variety that were successfully and formally distributed to Vietnamese farmers. Only mutant types are used to generate 15% of the rice and 50% of the soybean. Giza-176 and Sakha-10 are high yielding rice mutants produced in Egypt, Gines is a rice mutant produced by proton irradiation in Cuba, which thrives well in salty conditions, and further irradiating IR-5 rice in Myanmar gave a mutant "Shwewartun" which matures early with better yield and good grain yield.

In India, 200 Gy gamma irradiation was used to create the Sharbati Sonara wheat, which has an amber grain colour, early maturity, and a high yield of protein. It received official approval in 1967 and was made available to farmers. This particular wheat type was crucial to India's green revolution. In Italy, thermal neutrons were specifically used to make durum wheat, a creso mutant. Whisky and beer are made from Golden Promise barley, a gamma-irradiated semi-dwarf and salt-tolerant mutant that was introduced in the United Kingdom. Two high producing mutant barley cultivars in the USA include Luther and Pennrad.

Source: Mutation Breeding. www.wikipedia.org

Mutant varieties of some important crops

	crop	**Mutant variety**
1.	Rice	Jagannath, Mohan, Padmani, Sattari, Pusa NR-571, Dhanu etc.
2.	Wheat	Sharbati sonara, NP-836 etc.
3.	Groundnut	TG-38, TG-39, GPBD-5 etc.
4.	Soybean	MACS-450, TAMS-98-21, NRC-12 etc.
5.	Cotton	MCU-10, MCU-7, Indore-2 etc.

In addition, a number of variations have developed naturally through mutations or as bud sports of preexisting types. "Pusa Christina," "Abhisarika," and "Madhosh," three rose varieties created at IARI through induced mutations.

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