AN OVERVIEW OF MUTATION AND CURRENT SCENARIO OF INDUCED MUTATION IN TERMS OF PLANT MUTAGENESIS

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ABSTRACT

Mutation is one of the most significant concepts of modern Biology as it deals with the alteration of genetic characters of any individual. Starting from the discovery of Mendel's laws of inheritance, the science of mutation has grown enormously. Mutation can be beneficial if a new useful character or metabolic cycle is gained; but it can be detrimental if a desired and useful character is lost. But this mutation, especially induced mutation can be extensively used in production of superior varieties of crops – it can enhance the quality, productivity and disease resistance characters of the plants. Hence mutation breeding, by applying physical and chemical mutagens, has become a recent trend to meet the huge demand of food worldwide. The present paper discusses about the general sources and effects of mutation, and specifically focuses on the successful application of induced mutation in terms of development of useful varieties by plant mutagenesis.

Keywords: - *Mutation, Induced mutation, Mutation breeding, Mutagens, Plant mutagenesis.*

1. INTRODUCTION:

A mutation is the permanent alteration of the nucleotide sequence and it occurs when a DNA gene is damaged or altered [1]. In 1901, a Dutch scientist Hugo de Vries coined the term mutation from his experiments on the evening primrose *Oenothera lamarckiana* [2]. Mutation plays a vital role in the path of evolution because it is the ultimate source for all the genetic variations that occur in the ecosystem. Mutation is basically the building block and the first step towards evolution. A new DNA sequence for a particular gene is created, thus creating a new allele [3]. Mutagen is the physical or chemical agent which brings about the permanent change in the genetic material of an organism, primarily DNA. Mutagens are also termed as Carcinogens as most of the mutations which occur cause cancer [4]. The process by which the genetic information of an organism gets altered is called mutagenesis. It can occur spontaneously or when it is exposed to some mutagens [5].

1.1 General Sources or Mechanisms of Mutation:

- *Insertion* – Here, a piece of DNA is being added to change the number of DNA bases.

- *Deletion* – A piece of DNA is being removed which in turn changes the number of DNA bases. A small deletion may remove a few base pairs in a gene whereas a larger deletion can remove an entire gene or its surrounding genes.

- *Nonsense-mutation* – Substitution of one amino acid with a different one takes place.

- *Duplication* – A piece of DNA is abnormally copied once or more than once.

- *Frame-shift* – DNA bases get added or removed which changes the gene's reading frame. This reading frame consists of 3 nucleotide bases which codes for one amino acid. Thus, this whole group gets shifted which changes the code for that specific amino acid.

- *Silent mutation* – Gives rise to a type of codon which codes for the same amino acid or a different one but the functionality of the protein remains the same and it does not affect the phenotype as well. [6]

- *Tautomeric shift* – Shifting in the bases of DNA takes place. Isomerization of a nitrogen base to an alternative hydrogen bonding form takes place, the bases shift between keto and enol form or amino and imino forms. [7]

- *Depurination* – The process by which the purine bases of a DNA molecule are lost, which leads to carcinogenesis and somatic mutation. [8]

- *Deamination* – Removal of amino acid from a molecule. If there is an excess of protein intake, the amino acids get broken down.

- *Oxidatively damaged bases* – Process of oxidative damage on the nucleotide bases, but mainly occurs for guanine due to its high oxidative potential as compared to the other bases. [9]

1.2. Results of mutation:

Somatic mutations have no effect on its offspring whereas for germ line mutation, it is passed on to the offspring. Germ line mutation can have a variety of effects –

- *No prominent change in the phenotype* – The mutation occurs at a stretch of DNA or a protein coding region, but it ends up not affecting the amino acid, thus not changing the protein.
- *Small changes* – Single mutation, which caused a cat's ears to curl backward.
- *Big changes* - Death of the organism may occur. Single mutation caused the insects to be DDT resistant.

Since all mutations are being dealt as bad, Mutations to the control gene can bring about a positive change. Just like in an orchestra, the conductor controls which member will play which part and at what rhythm, similarly a control gene regulates the expression of other genes. Example- The presence of Hox genes in many animals (Hox genes determine where the head and appendages go, thus helping in the buildup of limbs, eyes). So, evolving a prominent change in the body part may require a change in the Hox gene and natural selection.

Scientists have found mutated Hox genes where the appendages have grown in place of antennae on the forehead of such flies [10].

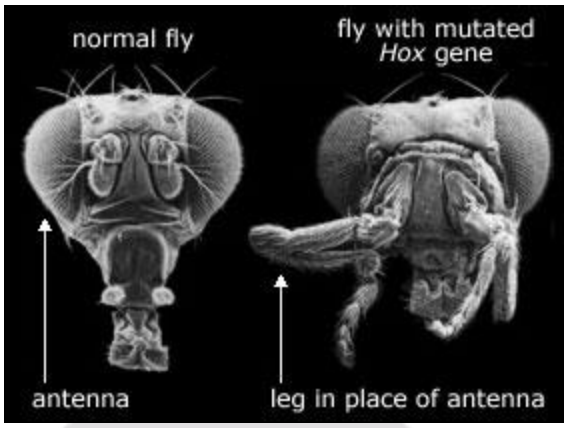

Fig.1. Mutation in Hox gene of fruit flies.

Some beneficial mutations-

- Some bacteria have mutated to survive in the presence of antibiotic drugs, which lead to antibiotic strains of DNA.
- A unique mutation has been found in an individual in Italy, that is this individual is resistant to atherosclerosis, which is basically a buildup of cholesterol plaque in the arterial walls which leads to blockage.

Some harmful mutations –

- If the mutation is germline, it is going to pass this disorder into future generations. Example- Cystic fibrosis (thick sticky mucous which blocks the lungs and the digestive ducts)
- Cells can grow at an abnormal rate and form masses of cells which is hereditary. Example- Cancer.

Bottomline - Mutations are important because it leads to evolution of an organism. It also causes genetic variation [11].

1.3. Types of mutation:

The DNA sequence of gene can be altered in a number of ways and a mutation is a permanent change in the DNA sequence. Following are some of the major types of mutation:

1. Forward mutation and reverse mutation,
2. Somatic mutation and amorphic mutation,
3. Spontaneous mutation and induced mutation.

Forward & Reverse mutation: A mutation that changes the wild type allele of gene to a different allele. The resultant allele can be dominant and recessive to the wild type. This is called forward mutation.

On the other hand, a particular type of mutation which helps a mutant allele to revert back to wild type is called reverse mutation. Following diagram showing the proper sequence of forward and reverse mutation:

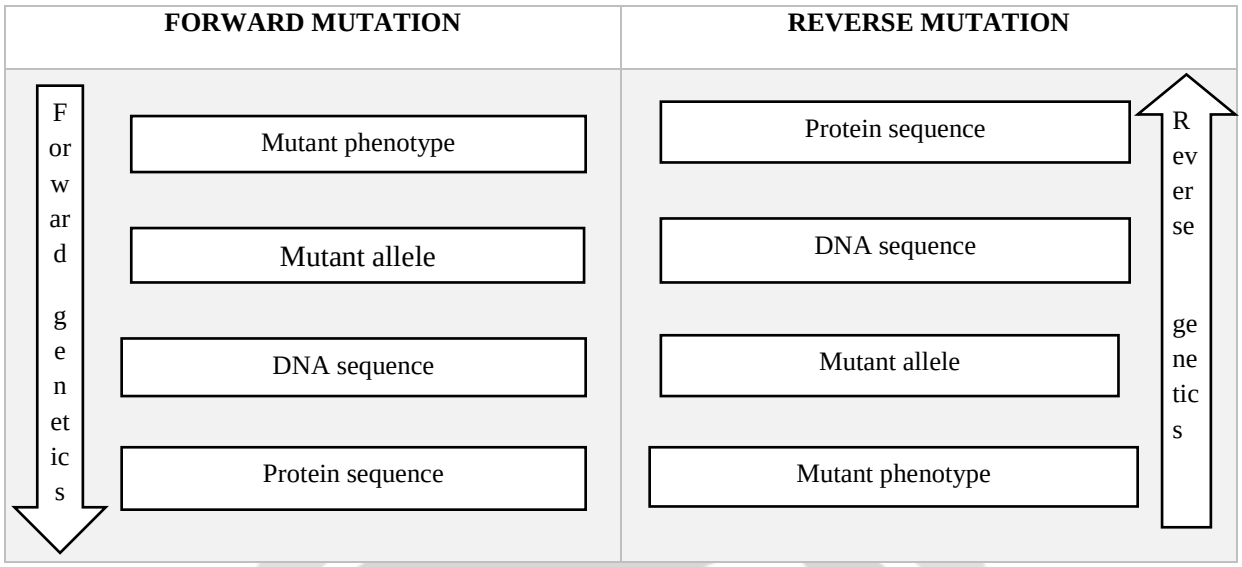

Chart 1: Diagram showing forward and reverse mutation

Somatic and Amorphic mutation: A change in the genetic mechanism that is not inherited from its parent genetic structure, and also not passed to the offspring is called a Somatic mutation. Mutation that occurs in germline cells is known as gametic mutation.

There is another kind of mutation where the parent allele loses the ability to encode any functional protein is called Amorphic mutation.

Causes of amorphic mutation: 1 Replacement of amino acids
2. Deletion of any part of enzymes

Types of amorphic mutation: 1. **Hypermorphic mutation**: It is the replacement of amino acids that would hinder enzyme activity.
2. **Hypomorphic mutation:** Helps in changing the regulation of the gene and causes it to overproduce than normal enzyme level.

Spontaneous and Induced mutation:

Spontaneous mutation is the type of mutation which arises naturally due to random changes in base sequence in DNA. It occurs at an average rate of approximately one in a million.

Induced mutation is any change in a particular DNA sequence or alternation in genes after it has come in contact with some external mutagens. It is basically a treatment with a physical or chemical agent which directly affects the DNA of a living organism.

Utilization: 1) plant breeding, 2) In increasing genetic variation.

2. MUTAGENS IN INDUCED MUTATION:

In genetics, a *mutagen* is an agent having the ability to change the genetic material, usually DNA, of an organism and thus change the frequency of mutations over the natural background [4]. Many mutagens are not mutagenic by themselves, but can form metabolites through cellular mechanisms, such mutagens are called *pro-mutagen*.

Example: 1) Cytochrome P450, 2) Cyclo-oxygenase.

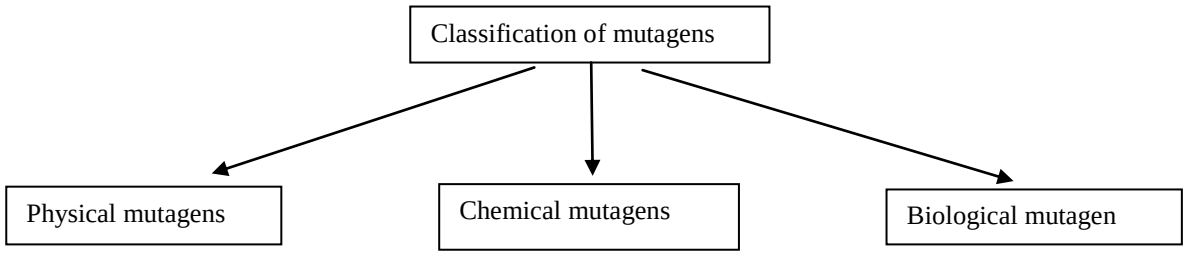

Table 1: Physical mutagens and their zone of action:

Physical mutagens or agents	Zone of action
X-ray	DNA breakage
Gamma ray	DNA breakage
Alpha particles	Chromosomal breakages
Cobalt-60	Rearrangement of chromosomes
Cesium-137	Translocation
UV- Rays	Produces pyrimidine dimers, error during replication

Table 2: Chemical mutagens and their zone of action:

Chemical mutagens or agents	Zone of action
Reactive oxygen species (ROS) (Includes superoxides; hydroxyl radicals; hydrogen peroxide)	Create problems in cellular processes. Abnormal electron transport.
Hydroperoxyeicosatetraenoic acid	Influence the breakage of DNA molecule by functional electrophiles.
Nitrous Acid	Helps in transitional mutations by converting cytosine to uracil.
Ethylnitrosourea (Alkylating agent)	DNA crosslinking & breakages.
Nitrosamines	DNA crosslinking
Aromatic amines	Influence carcinogenesis

Bromine	Make changes in chemical structures
Psoralen	DNA crosslinking
Sodium azide	Creates problems in organic synthesis

Table 3: Biological mutagens and their zone of action [1]

Biological mutagens or agents	Zone of action
Transposon	Insertion in chromosomal DNA disrupts functional elements of genes.
Bacteria	DNA damage, reduces the efficiency of DNA repair system.
Virus	Disrupts genetic function

2.1. Progression of human cancers through induced mutation technique

Mutations are the frequent cause of cancer. They are mostly viewed as independent events distributed randomly across chromosomes. There are basically two main reasons behind the progression of human cancers which can be explained through the induced mutation technique.

DNA strand breaks and crosslinking through mutagens: 15-hydroperoxyeicosatetraenoic acid, which is a cellular cyclooxygenase and lipoxygenase, breaks down to form 4-hydroxy-2(E)-nonenal, 4-hydroperoxy-2(E)-nonenal, 4-oxo-2(E)-nonenal, and cis-4,5-epoxy-2(E)-decanal. These bifunctional electrophiles are mutagenic in mammalian cells and helps in progression of human cancers. [13]

Mutation spectra: Mutation spectra directly indicates the clusters in cancer genomes which is caused by a subclass of apolipoprotein B mRNA-editing polypeptide-like cytidine deaminases (APOBEC) . These enzymes function to restrict retroviruses and retro-transposons by converting cytidine to uridine in single-stranded complementary DNAs (cDNAs). [14]

3. MECHNISM OF INDUCED MUTATION:

There are three mechanisms that induced mutation can follow:
1) Base analogue
2) Specific mispairing
3) Intercalation

3.1 Base Analogue:

DNA consists of the four type of bases Adenine(A), Guanine(G), Cytosine(C) and Thymine(T). When a chemical compound which is similar to these four base pairs gets substituted in the place of any one of these bases, it is called a base analogue. The binding properties of the base analogue is different from that of the naturally occurring nitrogen bases due to which incorrect nucleotides are formed during replication. The most common example for a base analogue is given by 5-bromouracil which like thymine is capable of binding with adenine with the difference being that bromine is the functional group in place of the methyl present with the 5^{th} carbon of thymine. The keto form of 5-bromouracil is capable of binding with adenine but the enol form binds with guanine. Thus 5-bromouracil if present in its enol form will bind with guanine and thus through replication will lead to formation of an incorrect G-C pair instead of the intended A-T pair. This results in mutation.

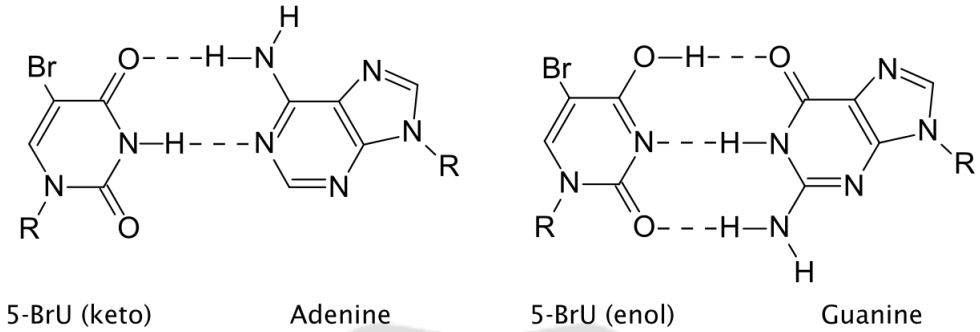

Fig.1. The keto-enol form of nitrogen bases [15]

3.2 Specific Mispairing:

When chemical compounds acting as mutagens do not get substituted in place of the four nitrogen bases but rather alters the structure of the bases by addition of alkyl groups, this leads to specific mispairing. An example of this is the addition of a methyl group to the oxygen attached to the 6th carbon of the nitrogen base guanine due to the activity of a mutagen such as Methylnitronitrosoguanidine (MNNG). This results in a mispairing as this guanine then binds with thymine instead of cytosine. This results in mutation as after replication an A-T base pair is formed instead of the intended G-C base pair. MNNG by the same mechanism can also act on the oxygen of the 4th carbon of thymine and add a methyl group. This leads to mispairing with guanine and after replication a G-C pair is formed instead of the intended A-T pair.

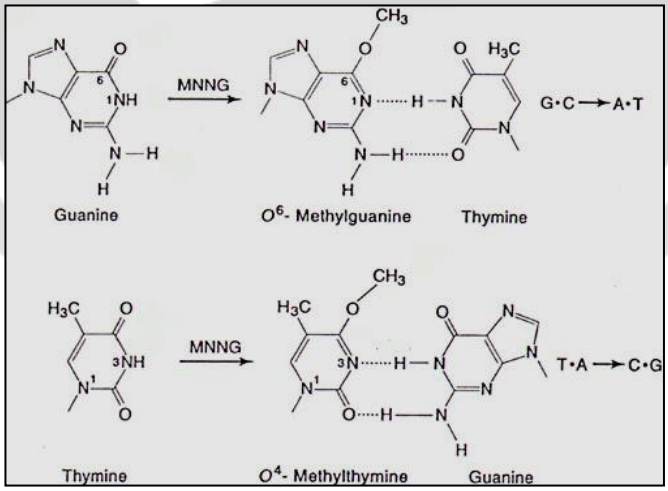

Fig -2: Specific mispairing by MNNG [16]

3.3 Intercalation:

Some compounds such as proflavin and acridine orange are capable of intercalating that is occupying the space between base pairs in the DNA sequence. This results in mutation as the intercalating compound causes structural changes in the DNA thus giving rise to functional changes such as insertion or deletion of single nucleotide pairs.

4. APPLICATIONS:

As described previously spontaneous mutations are rare in terms of occurrence and also random in nature, which makes them almost impossible to apply in various experiments for understanding any specific trait. But induced mutagenic mechanisms can be applied on various animal and plant species and even on microorganisms in a controlled manner. Mutation breeding is the most versatile field of application under this context as it helps in development of new varieties. Hence, induced mutation can be considered as the pillar of modern plant breeding and transgenic breeding technology [17].

4.1. Plant Breeding:

The most successful application of induced mutagenesis is in plant breeding which can be tracked back to 300BC when mutant crops were utilized in China [18]. But Muller's experiments on induced mutagenesis with physical mutagenic agents like X-rays on Drosophila flies showed a surprising 15000% increment in mutation rate which opened the path of mutation breeding [19].

4.2. Mechanism of plant breeding:

The process of mutagenic plant breeding begins with the selection of variety – the best variety of a crop having acceptable agronomic features like disease resistance, early growth and flowering etc. is always selected. These selected mutants are then re-evaluated under restrained and replicated environment to eliminate the false mutants. Depending upon the specific parts to be treated and specific desired traits required, different physical and chemical mutagens are selected [20].

The selected subjects are treated with particular mutagen taking mainly three things into account – the species upon which mutagenic treatment is to be given, dose or intensity of the treatment and duration. For the species which reproduces by forming seeds, the seeds are exposed to mutagens; similarly, for vegetatively propagated species, mutagenic treatment is applied on plant parts like buds, leaves, suckers or cuttings etc.

Regarding the dose of mutagen, a term 'LD50' is used which refers to the amount of mutagen that can kill half (50%) of all the treated individuals. The duration generally depends on the intensity of various radiation or concentration of mutagenic chemicals. This entire mechanism finally produces chimeras which are further examined whether the desired characters have been achieved or not.

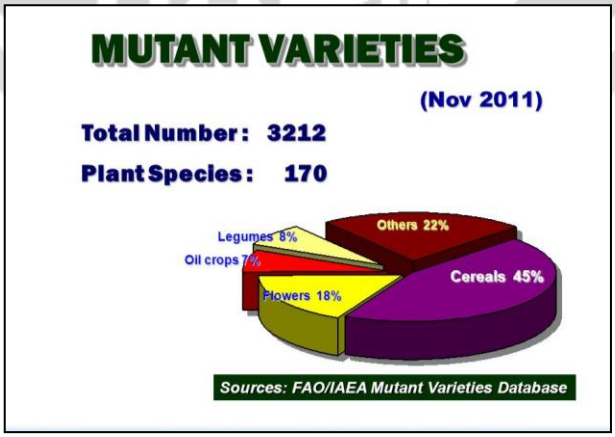

Fig.3. Diagrammatic representation of total number of mutant varieties of plant species (year-2011)

4.3. Benefits and recent scenario of plant breeding:

Mutation breeding can improve the genetic characters of vegetatively propagated plants which reproduce by asexual reproduction, and hence cannot be hybridized. Therefore, induced mutation is the only process that can improve these by apomixis. The first mutant generation or M1 plants are however heterozygous in nature as only one allele is affected by only one single mutation at the time of treatment [21]. An apomictic species shows sexuality for several generations. As a result, it can be hybridized normally using general hybridization methods.

With the help of induced mutation, it is now possible to have large varieties of species commercially available to meet the huge demand of food worldwide. It is now possible to produce desirable mutant alleles in the selected plant species. The productivity or yield of the crops can also be enhanced by this. Moreover, it can be used in overcoming self-incompatibility in plants.

The first mutant variety commercially produced was tobacco in 1934. In 1995, the total number of economically available varieties became 484 (principles). Within 2012, about 3248 mutant varieties were officially registered by Food and Agriculture. Asia leads the world with 1991 varieties (FOA). In the count of overall mutant variety production China (810 varieties) tops the list followed by Japan (481 varieties) and India. Within 2009, about 345 plant varieties were produced in India from 57 crop species. Several countries like China, India, Japan, Russia, USA, Thailand, Canada, UK, Germany, Pakistan, Bangladesh, Netherlands, Bulgaria etc. have already taken extensive programs for improvement of crop production and quality [22].

Applying induced mutagenesis, yield, maturity, quality, nutrition, functionality and tolerance to biotic and abiotic stresses have been improved so far. Numerous cultivars of wheat, maize, rice, cotton, mungbean, sesame, durum wheat, chickpea etc. have already been developed [23] Even, induced mutation is used for producing fruits like mango, banana, grapefruits, pear, apple, peach, mandarin, cherry almond etc. [24] and flowers like rose, marigold, dahlia, sunflower etc.

Table 4: Effects of different intensities of mutagens on the traits of crop species:

Crop species	**Mutagen used**	**Improved traits**	**References**
Sweet potato	γ rays (80Gy)	Improved salt tolerance	[25]
Sugarcane	γ rays (20-25Gy)	Mutants for agronomic traits	[25]
Musa spp. (banana)	Carbon ion beam (0.5 to 128 Gy)	Disease resistant	[25]
Chrysanthemum morifolium	gamma rays (25 Gy)	From red and white flower to development of yellow flower.	[25]
Rice	treatment of seeds in aerospace	Late maturity, medium tillering ability, panicle large multi-grain, high yield, better grain quality and resistance to blast	[26]
	gamma rays (100, 150, 200 Gy)	Semi dwarf stature, shorter duration, significant superior yield compared to the parent and retention of the basmati grain quality	[26]
	0.001M sodium azide (NaN_3)	Resistance to blast disease	[26]
	Carbon ion beams, 40Gy	High yield	[26]

	gamma rays, 700Gy	higher yield, less hairy stem, leaves and capsules, and tolerance to temporary waterlogged condition.	[26]
	NMU	Grain (seed) yield, enhanced nutrient quality.	[27]
Wheat	gamma rays (250 Gy)	immune to stripe rust, high yield, good quality and medium tillering ability.	[26]
	EMS	amylose free, lower pasting temperature, higher peak viscosity, and higher breakdown than for nonwaxy wheat.	[28]
	DMS, 0.004 M	Reduced plant height, early maturity, good resistance to diseases and high protein content	[26]
Maize	NMU, 0.001%	High yield and mid-late maturity, mutant variety for grain and silage	[26]
	200Gy Co 7-rays	strong disease resistance	[29]
	fast neutrons (7.5 Gy)	Improved combining ability	[26]
Barley	x-rays (100 Gy)	Erectoid type	[26]
	gamma rays (300 Gy)	Altered maturity and improved seed production traits	[26]
	NEU, 0.1%, 12Hours	Tolerance to low temperatures	[26]
	NEU, 0.04%	Improved lodging resistance	[26]
Groundnut	gamma rays, 200Gy	Waxy leaf, pod and seed size increased, seed coat color became brighter, moderately resistant to collar rot, CLS and rust, higher yield	[30]
Oat	gamma rays (400 Gy)	earliness, higher seed yield	[26]
	thN (thermal neutrons)	resistance to crown rust and high yield potential.	[26]
Soybean	gamma rays (100 Gy)	Earlier maturity	[26]
	gamma rays, 180Gy	drought tolerant, rush resistance, wide adaptability	[26]
	gamma rays, 140Gy	High yield, disease tolerance	[31]
Mustard	gamma rays, 2000Gy	High oil content, less erucic acid (11%), early maturity, high grain yield	[26]
	gamma rays, 700Gy	Shorter height, higher yield	[26]
Mungbean	EMS, 0.75%	Higher seed yield and synchronous pod maturity, tolerance to leaf YMV and Cercospora leaf spot	[26]
Rye	colchicine	Improved seed yield, seed size and vigor	[26]
Sunflower	Ultrasound, 25.2Other	High oil content and large number of seeds per head	[26]

	gamma rays, 120Gy	Large seeds, improved oil and protein content	[26]
Apple	gamma rays chronic	Altered leaf color	[26]
	gamma rays, 250Gy	plant structure and agronomic traits	[26]
Orange	gamma rays, 100Gy	Almost seedless, high yield and parthenogenesis	[26]
Lemon	x-rays, 10Gy	Improved fruits yield and fruits quality	[26]
Paper flower	gamma rays	Higher multiplication rate	[32]
Chrysanthemum	gamma rays, 40Gy	Enhanced flower colour	[26]
Rose	gamma rays	Light pink flower color instead of blend of light red and deep pink	[26]
	gamma rays, 75Gy	Pink stripes on a lemon yellow colored base of petals	[26]
Dahlia	gamma rays, 20Gy	Improved plant architecture, flower color (spirea red), ray florets are divided at the tips	[26]
	EMS, 0.6%, 13Hours	Reduction of internode length and plant height, increased fruit production	[26]

5. CONCLUSIONS

Mutations as explained yield great benefits and will continue to do so in the future with further improvements to the applications discussed earlier. However, there are some problems associated with the application of mutation. Mutations induced artificially are not completely controllable as although the intensity of the mutagens can be controlled, it is not certain that the mutation would lead to the desired effect. Again, even if the desired result is achieved to satisfy the needs of human beings, there is no certainty that along with the desired effect an undesirable effect will not develop. Such an undesirable effect may cause harm to the mutant. Changes in the DNA caused by induced mutation for crops may turn out to be pathogenic for the human beings consuming them. In spite of these limitations, induced mutation is quite successful in bringing in the new positive characters and producing superior crop varieties by enhancing the overall quality of the plants. It can be easily expected that in the near future more varieties of plants will be produced and at the same time modern technologies will improve the accuracy of induced mutation.

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