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A REVIEW ON MUTATION BREEDING FOR THE IMPROVEMENT OF CROP PLANTS: PRESENT AND FUTURE.

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ABSTRACT

Mutation, nowadays, has become an established tool in plant breeding and is generally used for developing crop cultivar/s or improving certain specific traits. An extensive work has been done for improving crop species by inducing mutations signifying their economic values. In the present work, two forms of mutation breeding i.e. natural mutations and induced mutations have been discussed. The natural mutations occur spontaneously and known to alter the structure of chromosomes in all plants, hence are harmful. While, induced mutations are generally grouped into two broad categories: chemical mutagens and physical mutagens. The physical mutagenesis is caused by UV rays, X-rays, cosmic rays, gamma rays, etc. and chemical mutagenesis is caused by EMS, colchicine, sodium azide, MNU, hydroxylamine hydrochloride, etc. Such genetic transformation by mutation breeding also creates morphological variants that act as a tool for understanding the physiology of the plants and optimistically, lead to their domestication. Presently, a number of mutants (chemicals and radiation) are being used to explore genomics, produce saturated genetic maps, for gene expression and regulation. Thus, mutation based breeding has been to renovate the well adapted plant varieties by attending one or two major trait/s even in *in vitro* culture, which limit their productivity or enhance their quality value.

Table 1: The list of mutant crops produced by X ray treatment.

Name of the crop	Traits improved	Authors
Glycine max	Increased oleic acid content	Rahman *et al.* 1994
Vigna unguiculata	Significant enhancement in length and fresh weight of shoot and leaf area	Ikram *et al.* 2015
Vigna radiata	*Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* were completely suppressed	Ikram *et al.* 2015
Triticum	Higher tolerance to adverse effects of saline condition	Asghari *et al.* 2013
Corchorus capsularis	Increase plant height	Sinhamahapatra and Rakshit 1990

Introduction*:*

Mutations play an important role in the evolution and considered as the chief source of origin of a new species or the creation of new genetic variation that the used in breeding programs. During the past few decades more than 2500 varieties have been released worldwide, which are derived either as direct mutants or from their progenies (Kharkwal *et al*. 2008). The prime strategy in mutation based breeding is to upgrade the well-adapted plant varieties by altering one or two major traits, which limit their productivity or increase their quality value. Further, the developed mutants can be used for morphological evaluation, molecular analysis, and even in studying phylogeny.

Presently, a number of mutants induced by chemical/s and radiation are being used to produce saturated genetic maps and explore genomics, gene expression and gene regulation (Ahloowalia and Maluszynski 2001). Several morphological and other mutant/s of the economic importance and academic interests have been isolated in different crops by various researchers and the work on mutation breeding has been reviewed (Kharkwal 1983; Khan and Farook 1998; Dixit *et al.* 2000). Thus, keeping in mind the successive use of mutational breeding, the present work has been compiled to understand the past and future of mutation breeding in crop plants.

Table 2: The list of various mutant crops produced by Gamma rays.

Name of crop plant	Traits	Authors
Legumes	Salt tolerant	Kumar *et al.* 2017
Carthamus tinctorius	Increase genetic variability	Kumar and Srivastva 2010
Abelmoschus moschatus	High yield	Warghat *et al.* 2011
Polianthes tuberosa	Higher genetic variability	Navabi *et al.* 2016
Arachis hypogaea	Increase grain yield and other morpho-agronomic parameters	Lukanda *et al.* 2013
Glycine max	Increase the genetic diversity and tolerance to drought	Aminah *et al.* 2015

Table 3: The list of various mutant crops produced by Colchicine treatment:

Name of plant species	Traits	Authors
Banana	Resistance to disease	Hamill *et al.* 1992
Pineapple	Resistance to disease, resistance to herbicides and antibiotics	Brar and Jain 1998
Kiwifruit	Increase fruit size	Wu *et al.* 2011
Brassica napus	Homozygous double haploid	Weber *et al.* 2004
Helianthus annuus	Rust resistance, height and maturity	Downes and Marshall 1983
Azuki bean	Increase seedling rate and plant survival rate	Pu *et al.* 2005
Sorghum bicolor BT×623	Longer leaf length and stronger root system	Murali *et al.* 2013
Raphanus sativus	Higher yield and wider adaptability	Manawadu *et al.* 2016
Prunella vulgaris	Superior tetraploid	Kwon *et al.* 2014

Types of Mutation Breeding:

The process mutagenesis involves sudden heritable change/s in the genetic information of an organism, not caused by genetic segregation or genetic recombination, but induced by chemical, physical or biological agents. Further mutagenesis may be categorized as: Natural and Induced mutations

1. **Natural mutations:** Such mutations occur spontaneously in all plants, by changing the chemistry of the plant genetic material or altering the structure of a chromosome. Most often the mutation is harmful and the change is erased by natural selection. Normaly, they are harmful or developing new crop cultivar/s and for changing the plan of traits caused by cosmic rays, which bombard the earth constantly, can penetrate matter readily. If one strikes a chromosome it can produce a mutational change/s. Normal physical phenomena, such as heat can cause mutations; so can pure oxygen under pressure. It has been shown that genetic material can mutate simply through the process aging. Natural evolution is based on three main factors: spontaneous mutations; hybridization, which reshuffles mutations into almost unlimited genetic patterns; and natural selection, which favours the perpetuation of individuals with genetic patterns which make them better able to survive and to reproduce.

Natural mutation happens randomly and therefore not easily traceable. There are various factors that cause these kind of mutations or increase their frequency. This includes the activity of mutagens found in nature, such as solar radiation or reactive chemicals such as depurinators or free radicals. Alterations during the replication of DNA during mitosis or unequal crossing-over events during meiosis can go unrepaired in a cell, leading to mutant progeny cells. It is difficult to find out that at what rate these mutations can occur, because the cellular machinery typically catches these mistakes. Occasionally, however, some alterations make it permanently into the organism's genome.

2. **Induced mutations**: On the basis of type of mutagen used, induced mutations are of two types: physical mutagens and chemical mutagens.

a. **Physical mutagens:** In the past 80 years, physical mutagens, mostly ionizing radiations, have been used widely for inducing hereditary aberrations and more than 70% of mutant varieties were developed using physical mutagenesis (Mba *et al*. 2013). Mutagenic radiations includes X–rays, ionizing particles and ultraviolet rays. The ionizing components of the Electromagnetic spectrum, include cosmic, gamma (γ) and X-rays (Mba *et al*. 2013). The most commonly used physical mutagens are X-rays. Since then, the various subatomic particles (neutrons, protons, beta particles and alpha particles) have been generated using nuclear reactors. Gamma radiations are mostly emitted by radioactive cobalt (^{60}Co) is mostly used (Acquaah 2006). However, it can be used for irradiating the whole plant and delicate materials, like pollen grains. Several mutant varieties have been developed through gamma radiation (Mba *et al*. 2010).

Ionizing emission goes deeper into the tissue and cause a great number of variations in the chemical composition. The major advantage of using physical mutagenesis compared to chemical mutagenesis is the degree of accuracy and sufficient reproducibility. During the past two decades, ion beams either through implantation or irradiation have become a new type of physical mutagen instead of the widely used gamma rays, X-rays and neutrons. The positively charged ions are accelerated at a high speed (about 20%–80% of the speed of light) and form high linear energy transfer (LET) radiation. LET radiation causes significant biological effects, such as chromosomal aberration, lethality, etc. as compared to other types of radiation used in physical mutagenesis. The damage caused by ion beams to DNA double strands is less repairable as compared to that induced by gamma rays due to deletion of DNA fragments of various sizes. More recently, to study the intricacies of mutation induction in space, plant materials have been sent out into aerospace. It has been speculated that the special environment of space flight, such as cosmic radiation, microgravity, weak geomagnetic field, etc. contains the potential agents of mutation induction. However, knowledge of the underlying genetics of aerospace mutagenesis is so far scarce (Mba *et a*l. 2012).

Ionizing radiations cause mutations by breaking chemical bonds in the DNA molecule, deleting a nucleotide, or substituting it with a new one also pointed out the importance of radiation being applied at the proper dose, a factor that depends on radiation intensity and duration of exposure. The exposure may be chronic (continuous low dose administered for a long period) or acute (high dose over a short period). The quality of mutation is not necessarily positively correlated with the dose rate. It is common knowledge that a high dose does not necessarily yield the best results. The mutagen dose used should be a compromise between a mutation load and the chance to find desirable mutations, and this greatly depends on the cost effectiveness of selection. Screening of larger mutant populations that originate from a lower mutagen dose may be feasible for traits with simple phenotypic selection criteria, such as early maturity. On the other hand, screening the same population for complex phenotypic traits, such as seed protein quality would not be feasible.

i. **X rays:** The first crop species to be mutagenized was barley by Stadler. In case of mutagenic barley, the resulting plants were white, yellow, pale yellow and some had white stripes. There are two novel approaches to barley mutagenesis. The most powerful approach is the use of neutron radiation for the production of deletion libraries. With this method, deletion in the range of 100-10,000bp can be generated. The second approach deals with transposons mutagenesis (Stadler *et al*. 1930).

ii. UV rays: The mutagenic effect of ultraviolet light was discovered by Altenbung (1934) through irradiation of the polar cap cells of fruit fly eggs. In those organisms, germ tissue could be easily exposed to the low-penetrating ultraviolet light which resulted in covalent dimerization of adjacent pyrimidine. Emission of UV light (250–290 nm) has a modest capacity to infiltrate tissues as compared with ionizing radiation. The UV rays mutants were successfully produced in few crops (Table 1).

iii. **Gamma rays:** Gamma rays have a shorter wavelength and therefore, possess more energy than protons. Few important improved varieties using gamma radiations are listed in the table 2.

iv. Neutrons: Neutrons are hazardous and hence have less penetrating abilities, but they are known to cause serious damage to the chromosomes. They are best used for materials, such as dry seeds. Various forms of neutrons were also studied extensively for their use in mutagenesis in the 1960s and 1970s. Though it has been proved to be an effective mutagen, particularly for producing large DNA fragment deletions, the application of neutrons in induced mutagenesis is limited. In *Capsicum annuum* higher yield is obtained by treating with neutrons (Falusi *et al*. 2012)

Table 4: The list of various mutant crops produced by sodium azide (NaN_3) treatment:

Name of plant species	Traits	Authors
Hordeum vulgare	Chlorophyll mutant	Prina and Fevret 1983
Pisum sativum	Pyridoxin deficient	Kumar 1988
Musa spp.	Resistant against *Fusarium oxysporum* f. sp. Cubense	Bhagwat and Duncane 1988
Hordeum vulgare	Anthocyanins and proanthocynidins deficient	Olsen *et al.* 1993
Lactuca sativa	Down mildew resistant	Okubara *et al.*1994
Avena strigosa	Disease resistant	Papadopoulou *et al.* 1999
Triticum aestivum	Salt tolerance	Agata *et al.* 2001
Oryza sativa	Reduced amylase content	Jeng *et al.* 2003
Oryza sativa	Auxin resistant mutant	Chhun *et al.* 2003
Hordeum vulgare (barley)	Mildew resistant	Molina-Cano *et al.* 2003
Vigna radiata L.	Quantitative traits	Samiullah *et al.* 2004
Lagerstroemia indica	Resistant to powdery mildew and leathery foliage	White and Carl 2004
Vigna radiata L.	Quantitative traits	Samiullah *et al.* 2004
Spathoglottis plicata	Improved floricultural significance	Roy and Biswas 2005
Oryza sativa	Enhanced yield	Jeng *et al.* 2006
Zea mays	Resistant against pathogen *Striga*	Kiruki *et al.* 2006
Arachis hypogaea	Yield traits	Mensah and Obadoni 2007
Saccharum officinarum	Red rot (*Colletotricum falcatum*) resistant	Ali *et al.* 2007
Arachis hypogeal	Disease resistant	Mondal *et al.* 2007
Oryza sativa (rice)	Silicon deficient mutant	Nakata *et al.* 2008
Halianthus annuus	Reduced triacylglycerol	Venegas-Caleron *et al.* 2008
Halianthus annuus	Enhanced stearic acid content	Skoric *et al.* 2008
Oryza sativa L.	Enhanced amylase content	Suzuki *et al.* 2008
Zea mays	Drought tolerant mutant	He *et al.* 2009
Phaseolus vulgaris	Higher antioxidant activity	Jeng *et al.* 2010

b. Chemical mutagenesis: The chemical mutagenic agents have the main advantage that they can be applied without complicated equipment or facilities. For chemical mutagens the ratio of mutational to undesirable modifications is generally higher than for physical mutagens. For inducing mutations the material is soaked in a solution of the mutagen. During the application of chemical mutagens, the care should be taken as it is carcinogenic. Material and safety data sheets for the specific chemical mutagen should be chosen carefully and after the use of chemical agent it should be appropriately inactivated before disposal. From the large number of mutagenic compounds, only a few numbers have been tested in plants. Among them, only a few group of alkylating agents has shown large application in plant experimental mutagenesis and plant mutation breeding. About 80% of the registered new mutant plant varieties reported in the International Atomic Energy Association (IAEA) *database* achieved via chemical mutagenesis were induced by alkylating agents. Of these, there are compounds namely ethyl methane sulphonate (EMS), 1-methyl-1-nitrosourea which account for about 64% of these varieties are (Leitao *et al*. 2012).

Alkylating agents are common among a large group of classes of compounds, including sulphur mustards, nitrogen mustards, epoxides, ethyleneimines, ethyleneimides, alkyl methanesulphonates, alkylnitrosoureas, alkylnitrosoamines, alkylnitrosoamides, alkyl halides, alkyl sulphates, alkyl phosphates, chloroethylsulphides, chloroethylamines, diazoalkanes, etc. Alkylating agent is one of the most effective chemical mutagenic group (these agent react with the DNA by alkylating the phosphate groups as well as the purines and pyrimidines). Second group is that of the base analogues (they are closely related to the DNA bases and can be wrongly incorporated during replication) e.g. 5-bromouracil and maleic hydrazide. The point mutations created by chemical mutagens have great advantage to generate not only loss-of-function but also gain-of-function phenotypes, if the mutation leads to a modified protein activity or affinity, like tolerance to the herbicide glyphosate or sulphonylurea shown in *Medicago truncatula*. The effect of efficiency of mutagen is effected by the concentration of mutagen, the length of treatment and the temperature at which the experiment is carried out.

i. Colchicine: Colchicine is an alkaloid from *Colchicum autumnale*, which is widely used for the induction of polyploidy plants. It is the most effective and the most widely used treatment for chromosome doubling. It has been used with great success in a large number of crop species belonging to both dicot and monocot groups (Table 3).

ii. Sodium azide: It is well known that sodium azide induces chromosomal aberrations only at a very low rate compared to other mutagenic treatments. Sodium azide was induced for the first time in Barley (Gruszka *et al*. 2012) for creating mutants, and after that, a number of crops have been produced by sodium azide induced mutation (Table 4).

iii. EMS: Ethyl methanesulfonate (EMS) is a stable and effective chemical mutagen (Table 5).

iv. MNU: A chemical, 1-methyl-3-1-nitrosoguanidinenitro, is carcinogenic, but also used as a mutagen in breeding programmes (Table 6).

v. Hyroxylamine hydrochloride: Hydroxylamine can be used to mutagenize purified DNA. It results in C to T and G to A transition mutations when double stranded DNA is mutagenized. Hydroxylamine decomposes in hot water and can be neutralized in this way. It also decomposes in atmospheric moisture and carbon dioxide and should be stored desiccated at room temperature (Table 7).

A compilation of all the information gathered from different research articles published revealed that about a large number of crops varieties, like cereals (350 varieties), Legumes (62 varieties), Fruits (40 varieties), and about 462 varieties of ornamentals plants have been produced globally. Similarly, in India, 24 varieties of Rice, 12 of Barley, and 8 of cotton and groundnut have been produced as a result of mutational breedings.

In spite of successful implementation of induced mutations in 20th century, it was removed from the main stream of crop improvement programme. A number of breeding programme have been declared closed in the last two or three decades, which affects the general crop improvement programme. However, it has become a mainstream crop improvement method once again and significant efforts have been made to invest in few interventions. These efforts include: to promote new plant breeders, allotment of new research projects, and considerable investment in training and infrastructure. Besides, induced mutation, there is also a need for the use of cell and molecular biology techniques to enhance/ or better understanding the mutational events. Further, in the ongoing induced mutation, the use of pre-breeding and integration of phenomics, are also suggestedd for the improvement of crops.

Table 5: Various mutant crops produced by EMS treatment.

Name of plant species	Trait	Authors
Vigna radiata	Highest callus proliferation.	Rafiq *et al.* 2012
Gossypium hirsutum	Drought tolerance	Witt *et al.* 2016
Cucumis sativus	Homozygous mutant lines	Wang *et al.* 2014
Oryza sativa	Lethal dose determination	Talebi *et al.* 2012
Linum usitatissimum	High yielding variety	Rafiq *et al.* 2017
Ipomoea batatas	Salt tolerant	Luan *et al.* 2007
Trigonella foenum-graecum	Higher seed yield	Basu *et al.* 2008

Table 6: Various mutant crops produced by MNU treatment

Name of the crop plant	Traits	Author
Rice	Leaf emergence	Suzuki *et al.* 2008
Rice	High Glutelin content	Satoh *et al.* 1979
Barley	High chlorophyll content	Kurowska *et al.* 2012

Table 7: Various mutant crops produced by hydroxylamine hydrochloride treatment

Name of the crop plant	Traits	Author
Phaseolus vulgaris	Change in number, aerial biomass and pods per plant.	Mosisa *et al.* 2014
Sesamum indicum	Plant height and internode length increased	Birara *et al.* 2014

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