History of Plant Mutation Breeding and Global Impact of Mutant Varieties

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Abstract

Hugo de Vries coined the term 'Mutation' in his classical hypothesis known as 'Mutationtheorie'. Immediately after the discovery of mutagenic effects of X-rays on Drosophila by Muller and barley, maize by Stadler, about nine decades ago extensive experiments on induced mutations were initiated. In the three decades that followed the pioneering work of Muller and Stadler to understand the nature of mutations, induction techniques and their role in evolution, genetics and plant breeding, a great deal of work on basic aspects of induced mutation technique in understanding the mechanism of gene mutations, mode of action of physical and chemical mutagens was done world over. Several countries took up the task of crop improvement through the use of mutation technique in their classical breeding programmes as well as through molecular approaches. During 1950–1960, several countries took up the task of crop improvement through mutation breeding approaches, particularly after the establishment of the International Atomic Energy Agency (IAEA) which started coordinated programmes on the use of mutation breeding technique in a large number of crops in several countries of the world. Over 3500 mutant varieties belonging to >240 plant species including cereals, pulses, oilseeds, vegetables, fruits, fibres and ornamentals that have been developed and released by 2022 are evidence of the successful use of mutation technique in plant breeding. A wide range of characters including yield, flowering and maturity duration, plant architecture, quality and tolerance to biotic and abiotic stresses have been improved in the mutant varieties developed so far. As per the IAEA records, majority of these mutant varieties were developed and released as direct mutants, the rest were released through cross-breeding with mutants. Most of the mutant varieties have

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S. Penna, S. M. Jain (eds.), *Mutation Breeding for Sustainable Food Production and Climate Resilience*, https://doi.org/10.1007/978-981-16-9720-3_2

been developed using physical mutagens, with gamma rays alone accounting for the development of majority of the mutant varieties. Since induced mutagenesis is gaining importance in plant molecular biology as a tool to identify and isolate genes and to study their structure and function, interest in mutation techniques and mutation breeding has increased recently in several area of biological research. These studies have an enormous potential for future crop improvement programmes. To redesign our crops by placing important traits on genetic maps and to equip them with the genes and attributes that could meet the huge food production challenges, there is an urgent need to use a combination of molecular and induced mutation techniques. Large-scale use of mutation breeding methods have made a significant contribution to the national economies of several countries.

Keywords

Mutation · Mutagen · Mutation breeding · Mutant variety · Gamma rays · X-rays · Crop improvement

2.1 Introduction

A heritable change in a genetic characteristic of an organism caused by mutation is a natural process that creates new variants (alleles) of genes. Mutation is the ultimate source of all genetic variations existing in any organism, including plants. The variation so created by mutation provides the raw material for natural selection and is a driving force in evolution. This variation created by mutation is further amplified by recombination of alleles on homologous chromosomes and their independent assortment. No mutations-no evolution is an established fact, as mutations create new genes and, in this way, provides the raw material for natural selection to act on. Natural selection operates to bring about evolution of new races and species through the variability created by spontaneous natural mutations and amplified by subsequent recombination of genes during sexual reproduction. Mutations arising spontaneously are random events in terms of the time of their occurrence and the gene in which they occur. In this way, mutant forms showing both large and small effects on the phenotype arise for all kinds of traits. Many of the lethal mutations may make the organism less adapted to its environment. Others may confer no immediate advantage but may help to generate a wide range of useful recombinant genotypes through the subsequent process of independent segregation and crossing over of genes. Besides natural mutations that occur spontaneously due to various kinds of radiations and cosmic rays received from the sun and also emitted by several radioactive elements on the earth, mutation can also be artificially induced by a number of physical agents like gamma rays and X-rays and several types of chemical agents known as chemical mutagens.

Historically, it was in 300 BC in China that the first story of mutants in crop plants was described in the book *Lula*. However, the first natural mutant plant in cereals was found about 2317 years ago in China (Van Harten 1998). The earliest recorded history of a practical example of dominant germinal mutation in domestic animals and its use in cross-breeding is traced back to 1791, when an English farmer Seth Write noted an unusually short-legged male sheep in his herd at his farm by the Charles River in Dover, Massachusetts in the USA. This mutant sheep was further used to develop a short-legged breed of sheep named 'Ancon' (Van Harten 1998). Charles Darwin (1859) in his historical book *Origin of Species* postulated that variations are created spontaneously in nature, and these naturally created variations are responsible for the development and origin of different species from a single common ancestor. Darwin reported that only those genotypes that create variations and adapt themselves to the environmental condition prevailing will alone survive, and he called this phenomenon as *survival of the fittest by natural selection*. He further reported that these variations are heritable from one generation to another. He called these factors that cause variation as *sports* or *bud variation* produced *spontaneously* by unknown causes that play a major role in speciation. Darwin did recognize that mutant forms arose suddenly and spontaneously, but he was doubtful about their role in evolution. Although Darwin originally used the word *transmutation*, he however, narrowly missed the discovery of the concept of *mutation* in his theories as he largely rejected *discontinuous variations* as significant factors in evolution.

The fact that natural selection was a necessary condition for evolution was recognized by Bateson (1894), but he also did not believe that it was a sufficient explanation, primarily because so little was known about the facts of heritable variation. Bateson published his monumental 598-page treatise *Materials for the Study of Variation, treated with special regard to Discontinuity in the Origin of Species* in 1894. Bateson's *Materials* treatise bears a curious resemblance to Darwin's Origin of Species. Bateson tended to assume that there was a one-to-one relationship between unitary genetic factors and their associated heritable traits. In 1905, Bateson coined the word 'genetics' to describe and establish a new scientific academic discipline.

The genetic behaviour of variegation in the flowers of *Antirrhinum*, the snapdragon was studied by Hugo de Vries (1889). Here the flowers are white or yellow, with red stripes, the variegation being recessive to the self red condition. On a variegated plant, the size of the red areas is variable, and sometimes includes an entire branch. He showed that the flowers on those red branches behave like the red flowers on a wholly red F_1, that is, on self-pollination they give 3 red:1 variegated. The results were not given a Mendelian interpretation because, at the time the experiments were done, de Vries did not know Mendelism. He did, however, suggest that some kind of segregation was occurring, so that self colour was somehow split off from the variegated element and that no true whites were produced.

2.2 Discovery of Mutations

While experimenting for rediscovery of Mendel's laws of inheritance, Hugo de Vries in the late nineteenth century found variation in evening primrose (*Oenothera lamarckiana*) that doesn't follow mendelian pattern of inheritance (3:1), but it was heritable. Mutation as a mechanism of creating variability was first identified by Hugo de Vries in 1901, and he considered them as heritable changes by mechanisms distinct from recombination and segregation. Credited with the discovery of mutations, de Vries (1901, 1903) described them as suddenly arising changes in the organisms, which were inherited and produced relatively large effects on the phenotype. He coined the term 'mutation' and presented an integrated concept concerning the occurrence of sudden, shock-like changes (leaps) of existing traits, which led to the origin of new species and variation. He also clearly included in this term mutations with 'small effects'. In his experiments with evening primrose, de Vries observed many aberrant types, which he called 'mutants'. The notion of mutation which was used by de Vries to indicate sudden genetic changes as a major cause of evolution quickly became established after the publication of his great 'Mutationstheorie' (1901–1903) in German. de Vries's work was soon translated in English and appeared as 'Species and Varieties, Their Origin by Mutation'. A great service was rendered by de Vries in 1900 in the Netherlands, along with Carl Correns (1900) in Germany and Erich Tshermak (1900) in Austria, as they simultaneously and independently re-discovered Mendel's long forgotten work. de Vries also had suggested that the new types of radiations like X-rays and gamma rays, discovered earlier by scientists like Conrad von Roentgen (1895), Bequerel Pierre and Marie Curie (1898) might be applied to induce mutations artificially. Because of the 'Mutation Theory of Evolution' based on the concept of mutations as the source of genetic variation and his early ideas about their potential use and value for plant breeding, de Vries's work around the turn of the century may be marked as the starting point of the history of mutation techniques in the discipline of plant breeding. The role of mutations in breeding was, however, first realized by H. J. Muller (1927) and Stadler (1928) through their X-rays experiments with fruit fly (*Drosophila melanogaster*) and maize and barley, respectively, for producing genetic changes.

In the early nineteenth century, there was little to be said about the cause of mutations except that they were rare, sudden, and discrete events that cause 'genes' as Johannsen (1909) called them to pass from one stable state to another. In his experiments with common bean, Johannsen (1913) described not only spontaneous drastic mutations, but also small mutations affecting the seed index (seed width versus seed length proportion). This is a character which closely falls into the class of continuous variation, and hence Johannsen may be regarded as the first who really proved the existence of spontaneous small mutations. Baur (1924) also emphasized repeatedly the importance of small mutations, which he called 'Kleinmutationen', majority of which exert only slight effects. The difference in Darwinian evolution in plant and animal breeding are not so different as the natural selection is substituted by human selection in the hybrid progeny in the course of breeding.

2.2.1 Muller's Discovery of Induction of Mutations on Drosophila

The early work of Thomas Hunt Morgan (1910, 1912) and C. B. Bridges (1916) on mutations on Drosophila, furnished several instances of the occurrence of new dominant genes, and many new sex-linked recessives, in pedigree material. These examples confirmed the conclusion that mutation occurs in a single gene in a single cell, and that it can occur at any stage of development. However, as these results were merely qualitative, since the frequencies were too low for a quantitative study and were also strongly influenced by the personal equation of the observer, an objective index was needed so that one would recognize a class of mutations that was frequent enough to give significant numerical values. Both of these requirements were met in the elegant mutation techniques devised by Muller for the study of newly arisen sex-linked lethals in Drosophila.

Muller's mutation technique depended on the study of the sex ratios from individual females that were heterozygous for sex-linked 'marker' genes. This technique made possible an objective and unambiguous determination of the frequency of the sex-linked lethal. Muller published a brief report in Science (Muller 1927) on the use of mutation technique to establish priority for his discovery of X-ray-induced mutagenesis on fruit fly (*Drosophila melanogaster*) before leaving for Berlin in Germany to attend the fifth International Congress of Genetics (ICG) to present the very first paper on X-ray-induced mutagenesis technique. It appeared with the sensational title 'Artificial transmutation of the gene'. This was an important discovery with far reaching implications both for genetics and for crop improvement. The first paper on the use of mutation technique and the discovery of induced mutagenesis presented at the International Congress of Genetics, Berlin was Muller's first comprehensive report on his X-ray work on fruit fly (*Drosophila melanogaster*). His pioneering work, which opened a new era in genetics and plant breeding, was greatly appreciated. Muller presented the elegant mutation techniques that he had developed for scoring mutations in Drosophila and in determining their rates. He reported that mutation rates of sex-linked recessive lethals could be greatly increased in Drosophila following treatment of sperms in male flies with high doses of X-rays. It was his technique, which showed that genes generally show a mutation rate of 10^{-5} to 10^{-6} per locus per generation. This means that one out of a 100,000 copies of the wild type gene can mutate in the course of one generation. He also introduced the concept of generation time while considering mutation rates in different organisms. Muller's discovery of induction of mutation and rates of mutations, a breakthrough in the history of genetics, has contributed enormously to our understanding of genes and their evolution.

2.2.1.1 *ClB*: An Elegant Technique

Muller (1930) then improved the mutation technique by using the 'ClB' chromosome that he found in his experiments. This is an X chromosome that carries a crossover reducer, a lethal, and the dominant mutant gene 'Bar'. As is well known now, this chromosome made it possible to detect new sex-linked lethals without anaesthetizing the flies or make counts—merely by rapid examination of individual culture bottles. Through this technique one could test many more chromosomes than possible ever before and thus could obtain adequate data on the lethal frequencies. Crosses were made in the earlier mutation experiments between flies containing a different collection of genes in their X-chromosomes. In most cases, only one parent was subjected to the X-ray treatment. The female offspring from these crosses would contain any new mutant gene that might have arisen in only one of their X-chromosomes, and hence they would manifest no abnormality if, as usual, the mutant gene were recessive.

A major difference between the control and treated series was soon observed when the offspring (‘F_2’) from the above cultures were examined. The controls showed a very low frequency of lethal mutations (1 in 947 fertile cultures), like that usually found, whereas the treated series showed a surprisingly high frequency of lethals (88 in 758 cultures), and all but three of these lethals were confined to the chromosomes derived from the treated progenitor. Similar results were found with regard to visible mutations and semi-lethals in these cultures though of course they were not as numerous as the lethals. In later generations (‘F_3’ and ‘F_4’), only a small number of new mutations were found, not significantly more in the treated than in the control series. When Muller applied his ‘ClB’ technique to the study of gamete treated with X-rays, it was apparent that there was a marked increase in the frequency of newly arising lethals. This is the clear example of the use of mutation technique for artificial induction of mutations.

2.2.2 Stadler’s Discovery of Induction of Mutations in Plant Systems

The discovery of mutagenic action of X-rays demonstrated in maize, barley and wheat by Lewis John Stadler in 1928 and 1930 started the use of mutation technique for generating novel genetic variability through radiations in plants also. Stadler had begun his studies with barley at about the same time that Muller’s work with X-rays on Drosophila began, but since he was using an annual plant, his results were not available until after Muller’s papers were published. His first paper did not furnish independent confirmation of Muller’s result, on a different material studied with a different mutation technique. Although Stadler also published data on induced mutations in barley, he described them with much pessimism, stating that they would be of no use for plant breeding (Stadler 1928). By 1930, Stadler had obtained X-ray mutations by hundreds. Stadler reported the production of solitary mutations and an increase in lethality by X-rays. His experimental designs were elegant and critical, and the dimensions most impressive. Many geneticists believe the induction of mutation in plants as a breakthrough in the history of genetics and plant breeding.

2.3 Early Experiments with Induced Mutations

The exhaustive results of the mutation technique used by Muller and of Stadler at last opened the way to an extensive experimental study on induced mutations that is still being actively practiced. A great deal of work on basic aspects of induced mutation technique in understanding the mechanism of gene mutations, mode of action of physical and chemical mutagens was done world over to understand the nature of mutations, induction techniques and their role in evolution, genetics and plant breeding.

Hanson and Heys (1929) studied the induction of lethals in Drosophila by using radium mutation technique. They interposed lead shields of different thickness and recorded the ionization in each treatment. The curves for ionization and for mutation rate were superposable, leading to the conclusion that ionization is responsible for the mutations and that the relation is a simple, direct one—a 'one hit' phenomenon. Oliver (1930) confirmed this conclusion by showing that varying the dose by varying the duration of exposure to a constant X-ray source also gave a linear curve relating dosage to mutation rate. Muller showed that in the same year that the amount of background radiation is far less than would be required to produce the normal 'spontaneous' frequency of mutations. It came to be very generally accepted that the total ionization is all that need be considered in connection with radiation-induced mutations, at least within a strain.

Altenberg (1934) discovered the mutagenic effect of ultraviolet light (UV) through irradiation of the polar cap cells of fruit fly eggs. Stadler (1941) introduced a new mutagenic agent, ultraviolet light, in plants into his laboratory. The mutagenic potential of these rays has since been confirmed in many organisms in which germ tissue could be easily exposed to the low-penetrating UV. While the gamma radiation has become a very popular mutagen since the 1950s that has been used extensively to induce mutations, various forms of neutron had also been studied (Muller 1954) for their use in mutagenesis in the 1960–1970s though their application in induced mutagenesis has been limited.

2.3.1 Classic Examples of Early Application of Induced Mutations

The two major discoveries of the induction of mutations made by Muller (1927) in *Drosophila* and Stadler (1928) in maize and barley plants demonstrated that with the help of these physical mutagenic agents, it was possible to obtain mutation rates that were much higher than spontaneous rates. These discoveries lead to extensive works on induced mutations and showed the practical potential of radiation as a plant breeding tool and resulted almost immediately in the practical recovery of some economically useful mutants in wheat (Delaunay 1931; Sapehin 1930, 1936). Stubbe (1934) described 'small mutations' (now known as 'point mutations' or 'micromutations') for the first time in higher plants such as *Antirrhinum*. Knapp (1950) presented clear suggestions about how to utilize the micromutations in barley.

Tollenaar (1934) was the first worker to isolate a light green ‘chlorina’ mutant in tobacco which was released for commercial cultivation (Kharkwal 2012). The lecture delivered by Nilsson-Ehle on polymorphic factors in barley in Halle in the year 1939 opened a new era in the use of induced mutation in Germany. Timofeeff-Ressovsky (1941) reviewed his extensive work on X-ray mutagenesis techniques in Drosophila and his pioneering interpretation of mutagenicity data in terms of the classical target theory (Timofeeff-Ressovsky and Zimmer 1947; Zimmer 1961). Later, Freisleben and Lein (1942) reported the induction of mildew resistance in barley by X-irradiation. Mutation is not a directed process as is evident from the DNA structure which also suggested that mutations would occur in a random manner because basically they are unpredictable mistakes during the process of replication. Luria and Delbruck’s mutation technique in 1943 provided convincing evidence in support of random, non-adaptive nature of mutations in an experiment. According to their mutation technique, they counted the number of mutant individuals showing drug resistance in different cultures of wild type bacteria.

2.3.2 Gustafsson Builds Up the Momentum for Mutation Breeding

Swedish research on induced mutations in barley started in 1928 on a small scale at Svalöf, Sweden, initiated by the eminent Swedish geneticists Herman Nilsson-Ehle and Åke Gustafsson. The first treatments with irradiation commenced using the Svalöf cultivar ‘Gull’, which was the most common barley cultivar grown in Sweden at that time (Lundqvist 2009, 2021). A major stimulus for much of later work in further practical refinement of mutation technique and the usefulness of mutation breeding in crop improvement was demonstrated first in Sweden by the classic paper of Gustafsson (1947) which demonstrated practical plant breeding of agricultural plants by means of X-rays and ultraviolet rays induced mutations. Swedish plant breeders found many distinct categories of chlorophyll mutations like *albina*, *viridis* and *xantha* in barley by the use of X-rays. These chlorophyll mutations were always the first indication of treatment success (Gustafsson 1938, 1940), and their abundance served as the standard method for measuring the induced mutagenic effects. In the mid-1930s, the first viable mutations appeared, and it was possible to distinguish two subgroups: ‘Morphological’ and ‘Physiological’ mutations. They discovered some morphological mutants characterized by dense heads, late maturity and very stiff taller straw and resembled the *erectum* barleys, in comparison with the normal spikes in most of the barley cultivars. These mutants named *Erectoides* were found to yield higher and produce more straw than the maternal variety. A very large number of mutations of barley were shown to respond in a variety of ways in different genotypes (Lundqvist 2014). A variety of barley called ‘Pallas’ developed from stiff strawed and early mutants of the variety ‘Bonus’ was released for commercial cultivation in Sweden (Gustafsson 1963). These mutants represented the first actual accomplishments of the production of superior varieties by the use of radiation. Similar useful induced mutants reported included stem rust resistance caused by *Puccinia graminis* in wheat (MacKey 1954) and in oats (Frey 1955) and dwarf mutants in rice (Beachell 1957). The development of dwarf wheat and rice varieties that led to the green revolution are classic examples of mutation breeding achieved through successful exploitation of the natural mutant genes *Rht-1* and *Rht-2—Norin* in case of wheat and *sd1—dee-gee-woo-gen* in rice, which effect a large constellation of characters responsible for their superior agronomic responses and are called Green Revolution Genes.

2.4 Techniques for Detection and Analysis of Induced Quantitative Variation

Gaul (1961, 1965) classified mutations into 'macro' and 'micromutations' and used a systematic approach for the selection of micromutations in barley. Swaminathan (1963, 1965) in India broadly grouped induced mutations into four major types according to the degree of change in the phenotype and method of detection: (1) macro mutations, (2) mutations affecting single characters, (3) systematic mutations, and (4) micromutations. While estimating the induced variability due to micromutations, an increase in variance irrespective of character, symmetrical or skewed, has been the general observation while the mean mostly remained unchanged and sometimes even decreased. The hypothesis forwarded by Brock (1965) to explain such behaviour of induced mutations in quantitatively inherited traits is that random mutations are expected to increase the variance and shift the mean away from the direction of previous selection history. However, Gaul and Aastveit (1966) concluded that the mutations for quantitative characters are not related to the genotype, and with random mutation a change in the mean value of almost any quantitative character is to be expected. As a consequence of the nature of phenotypic manifestation of quantitative characters, the only method available to detect the induction of new variation for quantitative traits following mutagenic treatment is the estimation of mean and variance by statistical methods. The mean values of quantitative traits in populations derived from irradiated gametes (pollen) or embryos (seeds) are in most instances lower in the treated than in untreated populations. In a very extensive study, Scossiroli et al. (1966) reported a similar effect in the same population for a large number of characters. The effect of radiation on the means has been interpreted to be due to detrimental mutations which can be removed through selection in subsequent generations.

2.5 Discovery of Chemical Mutagenesis

After establishing X-rays mutagenesis in both animals and plants, numerous new mutagens, both physical like gamma rays, alpha and beta particles, neutrons, protons, ultraviolet (UV) radiation and chemicals like ethyl methane sulphonate (EMS), ethylineimine (EI), *N*-nitroso-*N*-methyl urea (NMU), sodium azide (SA), etc. were found to be effective in generating genetic variability. The technique of induction of mutations by chemical agents was attempted by many people over a long period, but until 1941 there were no clear and convincing positive results until it became established that certain chemicals could produce mutations. Auerbach (1941) was the first to report that mustard gas had a mutagenic effect on *Drosophila*, which was similar to that of X-rays on plants. Auerbach and Robson (1946) later obtained clear evidence that mustard gas is mutagenic.

The chemical mutagens were found to be highly effective in inducing true gene mutations and this specificity of action could be analysed in terms of the mechanism of their reaction with different DNA bases. The question, whether chemical agents do indeed produce mutations with the same frequency as the physical mutagens, was settled after the first paper published by Auerbach and Robson (1946). They used the standard mutation technique devised by Muller to score recessive and visible gene mutations in Drosophila following exposures of flies to a predetermined dose of the gas. Their most important observation was that mustard gas is highly mutagenic and capable of producing lethal and visible mutations at rates, which were comparable with the effect of X-rays. Besides gene mutations, chromosomal aberrations in the form of deletions, inversions and translocations were produced. In these diverse ways, mustard gas was found to be highly mutagenic. Auerbach (1949) presented her discovery of the mutagenicity of the 'radiomimetic' alkylating agent mustard gas at the ICG held in Stockholm in 1948. Oehlker (1943), and Gustafsson and Mackey (1948) also proved that mustard gas was mutagenic in barley as well.

Rapoport (1946, 1948) in Russia also discovered and demonstrated mutagenic effects of mustard gas and several other chemicals such as formaldehyde, diethylsulphate (dES), diazomethane, and other compounds and established that alkylating agents are the most important group of chemical mutagens.

2.5.1 Mechanism of Gene Mutation

The future direction of work on chemical mutagens was determined mainly by rapid advances in the understanding of gene structure and function following the demonstration of DNA as the genetic material. Watson and Crick (1953) while proposing their model for the structure of DNA pointed out that both replication and mutation of genes can be understood in terms of the new structure. Based on chemical studies nitrous acid was expected to convert cytocine to uracil and adenine to hypoxanthine, two of the known analogues of the nucleic acid bases. The modified bases mispaired during replication of the treated DNA leading to mutation through transitions. Freese (1959) reported extensive studies on reverse mutations resulting in wild type phenotype in rII mutants by two analogues—5-bromouracil (BU) and 2-amino-purine (AP), found to be highly mutagenic in viruses of the T4 series through their action in inducing A:T to G:C transitions.

The simplest kinds of base pair changes are due to transitions and transversions, but they can result in a phenotypically visible mutation. There are no restrictions on the different kinds of sequence changes in the DNA of a gene following different types of misprints during replication. Addition or deletion of a nucleotide base pair is another common error, when one of the bases manages to pair with two bases or fails to pair at all. These kinds of sequence changes resulting in an alteration in the reading frame of the gene's DNA are known as frameshift mutations. Some of the mutations occur from rearrangement of bases in the DNA. A small or large sequence of bases may be inverted as a result of chromosome breakage, and reunion of the broken ends may involve different DNA molecules in a reciprocal rearrangement or in loss of a fragment. Duplication of a DNA sequence is another common mechanism for change in the structure of a gene leading to gene mutation.

2.6 Application of Mutation Technique in Crop Improvement

A great deal of work was done world over on basic aspects of induced mutation technique in understanding the mechanism of gene mutations, mode of action of physical and chemical mutagens to understand the nature of mutations, induction techniques and their role in evolution, genetics and plant breeding (Kharkwal 2012). By now extensive use of mutation breeding has already established itself as a powerful plant breeding tool in its own right and made a significant contribution to food and fibre production and to farmer's incomes and national economies worldwide. Several countries took up the task of crop improvement through the use of induced mutagenesis technique and mutation breeding approaches during 1950–1970 and reported spectacular accomplishments of mutation breeding technique in evolving several superior crop varieties. Coordinated programmes were initiated by IAEA in a large number of crops in several countries of the world on the use of mutation breeding technique.

Significant contributions in understanding basic and applied nature of mutation phenomenon and techniques on cereal and legume crops were successfully made in several countries like Sweden (Gustafsson 1947, 1967 on barley), Germany (Gaul 1961, 1965; Gaul et al. 1969 on barley; Gottashalk and Wolff 1983 on peas), the USA (Konzak 1954 and Frey 1955 on oats, and by Gregory 1955; Gregory et al. 1960 on peanuts, Konzak et al. 1984), and India (Swaminathan et al. 1968 on wheat; Patil et al. 1995 on groundnut, and Kharkwal et al. 1988, 2005; Kharkwal and Shu 2009, Kharkwal 1998b, c on chickpea, lentil, pea and cowpea). Gregory et al. (1960, 1965) produced the first groundnut mutant variety NC 4x, about 5% better in total production in the USA by exposing about 200 pounds (90 kg) of peanuts to high doses of X-rays and planted one million M_2 plants in 64 acres in the university's crop development fields. Mutant var. NC 4x possesses between 400% and 500% fewer cracked pods than NC 2 and was released in January 1959 in the USA.

2.6.1 Ultra Modern Techniques of Mutation Breeding in Crop Improvement

The discovery of structure of DNA by Watson and Crick (1953) had brought a fundamental change in our understanding of gene mutations—transitions, transversions, frameshift mutations and misrepair mutagenesis, particularly caused by chemical mutagens. Recent advances in molecular biology have led to the establishment of several ultra modern techniques, which have helped researchers to redefine the scope, nature and applications of mutagenesis techniques. Induced mutagenesis technique is gaining importance as a tool in the identification of plant genes using molecular approaches. The main important established techniques are: (a) in vitro mutagenesis, (b) double haploid (DH) techniques (c) transposon-mediated mutagenesis, (d) site-directed mutagenesis (SDM), (e) random mutagenesis, (f) insertional mutagenesis, (g) systematic mutational analysis, and (h) targeting induced local lesions IN genomes (TILLING) (Colbert et al. 2001). With the recent advances in genomics, it has been documented that the use of high-throughput platforms, such as TILLING (Targeting Induced Local Lesions in Genomes) and EMAIL (Endonucleolytic Mutation Analysis by Internal Labelling) (Cross et al. 2008) in the rapid evaluation of mutant stocks for specific genomic sequence alterations can be very much helpful in studying the genetic variability at molecular level. With the advancement of molecular techniques, the mutation breeding comes into the era that is called the 'Molecular Mutation Breeding' in which molecular or genomic information and tools are used in the development of breeding strategies, screening, selection and verification of induced mutants, and in the utilization of mutated genes in the breeding process (Shu 2009). The latest rapid, high-throughput method in this field 'Mutagenomics'—the discovery of genes using the combination of mutants and genome sequencing (Hodgens et al. 2020)—is becoming increasingly useful in screening for point mutations. Some of the recently introduced ultra modern innovative techniques presently used for induction and study of mutation breeding parameters are briefly listed and introduced below.

2.6.1.1 High Hydrostatic Pressure (HHP)

Defined as an extreme thermo-physical factor that affects the multiple cellular processes like synthesis of DNA, RNA, proteins, cell survival (Ishii et al. 2004) and high hydrostatic pressure (HHP) has been very effective in inducing mutagenesis in microorganisms. The usage of HHP, a cost-effective technique in mutation breeding for creation of new mutant varieties has started since last few years only and one of the examples is the creation of mutant varieties of rice (Zhang et al. 2013).

2.6.1.2 Ion Beam Technology (IBT)

Ion Beam Technology was found to show high relative biological effectiveness (RBE) as compared to low linear energy transfer (LET) radiations, such as gamma rays, X-rays and electrons (Feng et al. 2009). Mutation breeding with heavy ion beams is unique technology wherein heavy ion beams are generated by accelerating atomic ions using a particle accelerator. Ion beams have been used to develop approximately 70 new crop varieties in Japan in the last two decades, and beneficial mutants have been identified in a variety of species (Abe et al. 2007, 2012, 2015, 2021).

2.6.1.3 Space Breeding Technology (SBT)

Space breeding technology, in which the growth cycle of the seeds could be shortened (Li 2013), has now become a proven way and can be applicable in modern mutation breeding strategies. The two parameters in combination i.e., presence of cosmic rays and microgravity, affect the genetic diversity of the crops in space and thus they are the main causes of the changes in breeding new crop varieties (Mei et al. 1998; Gu and Shen 1989). China is the leader in the field of space breeding, having more than 66 new varieties developed in the space breeding programme (Liu et al. 2005, 2021; Liu 2021).

2.6.1.4 Targeting Induced Local Lesions IN Genomes (TILLING)

TILLING is a method that allows directed identification of the mutations in a very specific gene. It is one of the most high-throughput, non-transgenic reverse-genetic approaches, which combines mutagenesis with a sensitive DNA screening technique and enables the recovery of individuals carrying allelic variants of candidate genes (Sato et al. 2006). It is an efficient early-screening tool for specific point mutations in genes of interest from a small population and enables geneticists to analyse gene function and associate genotype with phenotype. TILLING combines traditional mutagenesis followed by high-throughput mutation discovery, which can improve the efficiency of using induced mutations and nucleotide polymorphism discovery methods for a reverse genetic strategy that is high in throughput, low in cost and applicable to most organisms, and in less than a decade will help to develop crops with improved traits (Colbert et al. 2001; Till et al. 2009).

2.6.1.5 Endonucleolytic Mutation Analysis by Internal Labelling (EMAIL)

EMAIL has been developed by Cross et al. (2008) for detecting rare mutations in specific genes in pooled samples using capillary electrophoresis. EMAIL, is an alternate approach to mismatch detection, in which amplicon labelling is achieved by incorporating fluorescently labelled deoxynucleotides. This technique which is highly improved over TILLING, offers the plant breeder a new tool for efficient screening of induced point mutation at an early stage for variants in genes of specific interest before taking plants to field trials, offers increased sensitivity in gene-specific mutant detection in pooled samples, enabling enlarged pool sizes and improving throughput and efficiency (Lee et al. 2009).

2.7 Role of Mutation Breeding in Crop Improvement

The standard technique of creating variability by means of altering genes through induction of mutations by physical or chemical mutagens and using the same effectively through elaborate methods of selection techniques in various generations for improvement of a particular crop species for desired objectives is called mutation breeding and is frequently practiced by plant breeders all over the world for crop improvement. Results of mutation breeding were more often useful in self-pollinating plant species. Success has also been tremendous in ornamental plants and in vegetatively propagated crops, which usually are heterozygous. Today, mutation breeding for crop improvement is not based only upon classical physical mutagens like X- or gamma rays or classical chemical mutagens such as EMS or NMU, but also upon variation that occurs during in vitro culture (Suprasanna and Nakagawa 2012) and has been termed ‘somaclonal variation’. Use of haploids derived from anther culture has also found its best application in the ‘doubled-haploids-technique’, which leads faster to homozygosity for more effective selection (Shu et al. 2012).

The impact of induced mutation on crop improvement is reflected in the 3365 mutant varieties officially registered by FAO/IAEA (http://mvd.iaea.org) carrying novel-induced variation. Moreover, about three-quarters of these are direct mutant varieties derived from treatment with gamma rays, thus highlighting the importance of peaceful usage of radiations that belong to the group of physical mutagens. All this translates into a tremendous economic impact on world agriculture, poverty alleviation, food security and food production that is currently valued in billions of dollars and millions of cultivated hectares (Kharkwal and Shu 2009). A detailed description of the economic impact of a large number of prominent mutant varieties released in several countries of the world is given in Chap. 13 contributed by this author.

2.8 Development of Crop Varieties Through Mutation Breeding: Global Scenario

Ever since the discoveries of induction of mutations made by Muller and Stadler, a large number of breeders in several countries followed mutation breeding, induced and generated significant amount of genetic variability through various mutagens. Because of the popularity, easy and safe access, majority of the genetic variability has been induced through radiations, particularly gamma rays that have contributed significantly to modern plant breeding. Among the mutant varieties released, the majority are food crops and ornamentals. A detailed database on the mutation-derived varieties developed and released in major crops all over the world is being maintained by FAO/IAEA (http://mvd.iaea.org). The number of mutant varieties officially released at the beginning of the year 2022 is >3365 belonging to >240 plant species. However, as the Mutant Variety Database of IAEA is perhaps not being updated very regularly, as is evident from the number of mutant varieties released in China (817), India (341) and Pakistan (56) reported in the MVD, whereas China has already reported >1000 mutant varieties (Liu 2021), India has already reported 542 (Table 2.1) and Pakistan has released 79 mutant varieties (Table 2.1—T. M. Shah personal communication), the number of mutant varieties released in the world from >240 plant species is expected to be >3500. Majority of the mutant varieties have been released during the last three decades. The cumulative number of officially released mutant varieties in six continents of the world indicates that Asia tops the regional list with 2052 closely followed by Europe (960) and North America (209). With more than 100 mutant varieties each, China, India, Japan, Russian

Table 2.1 Number of mutant cultivars released in top 20 countries and top 20 crops of the world

No.	Country	No. of mutants	No.	Crop	No. of mutants
1	China	817	1	Rice	853
2	India	542[a]	2	Barley	311
3	Japan	479	3	Chrysanthemum	266
4	Russian Federation	216	4	Wheat	265
5	Netherlands	176	5	Soybean	181
6	Germany	171	6	Maize	96
7	USA	139	7	Groundnut	79
8	Pakistan	79[b]	8	Rose	67
9	Bulgaria	76	9	Common bean	57
10	Bangladesh	76	10	Cotton	48
11	Vietnam	58	11	Mung bean	39
12	Canada	40	12	Dahlia	36
13	South Korea	40	13	Durum wheat	31
14	France	39	14	Pea	34
15	Italy	35	15	Sesame	30
16	United Kingdom	34	16	Chickpea	27
17	Poland	31	17	Tomato	25
18	Sweden	26	18	Rapeseed	24
19	Guyana	26	19	Oat	23
20	Thailand	24	20	Faba bean	20

http://mvd.iaea.org (accessed in March, 2022)
[a] Detailed lists in Chap. 12 by this author
[b] Personal communication from T. M. Shah, Director, NIAB, Faisalabad, Pakistan

Federation, the Netherlands, Germany and the USA are the leading countries engaged in the development and release of mutant varieties. A perusal of the data on specific crops and number of mutant varieties released in the world (Table 2.1) indicates that the top 20 ranks are occupied by some of the most important food (cereals, pulses, oilseeds), fibre and ornamental plant species in the world agriculture and economics.

During the last five decades, several countries took up extensive crop improvement programmes through the use of induced mutagenesis and mutation breeding and made spectacular accomplishments in evolving several superior mutant varieties in large number of important agricultural crop species including cereals, pulses, oilseeds, vegetables, fruits, fibres and ornamentals. A wide range of characters including yield, maturity, quality and tolerance to biotic and abiotic stresses have been improved in the mutant varieties developed so far. Although an exact estimate of the total area covered by commercially released mutant cultivars in a large number of countries is not readily available, but they are being cultivated in millions of hectares and have made a very significant contribution worth billions of dollars in global agriculture addressing food and nutritional security problems in many countries of the world (Kharkwal and Shu 2009; Kharkwal 2017).

Most of the released induced mutant varieties belong to seed-propagated crop plants species and nearly 25% are of the ornamentals. Of the total mutant varieties, majority mutant varieties were developed 'directly' after mutagenic treatment and selection in the subsequent generations. The remaining new mutant varieties were developed 'indirectly' through cross-breeding of mutants or already released mutant varieties as sources of desired characters in cross-breeding programmes. Among the various mutagenic agents used for developing varieties, a great majority were obtained with the use of physical mutagens like radiations, particularly gamma rays as the mutagen.

Mutation breeding in rice has been very successful worldwide, probably due to its diploid nature and self-fertilizing character. Out of 1616 mutant varieties of cereals, rice alone accounts for 853 varieties. The first widely known rice mutant cultivar has been cv. Reimei, a short straw (semi-dwarf) mutant from cv. Fujiminori, released in Japan in 1966. Several important cultivars were developed thereafter through cross-breeding with this cultivar (Van Harten 1998; Nakagawa 2021). The mutant cultivar carried an allele that was allelic to the well-known *sd1* [semi-dwarf allele in cultivar *Dee-geo-wu-gen* (DGWG), a spontaneous dwarf mutant discovered by Chinese scientists]. This mutant DGWG possessing dwarfness, stiff straw, fertilizer responsiveness, non-lodging and day length insensitivity as its most important traits, was the forerunner of green revolution. It was used in crossing programmes for developing important cultivars like Taichung Native-1 and IR 8. In barley, 311 mutant varieties superior to best control lines for various plant characters have been isolated and released by various countries, including Sweden (Gustafsson 1963). Some of the important mutant varieties of barley are 'Pallas' (Sweden), 'Balder J' (Finland), 'Diamant' (Czech Republic), 'Trumpf' (Germany), 'Goldspear' (UK), 'Pennrad' and 'Luther' (USA) and 'Betina' (France) (Van Harten 1998).

2.8.1 Mutants in Recombination Breeding

Although majority of mutant varieties out of the total of 3365 reported were released as direct mutants and one third through crosses with various mutants, the trend of late is changing towards more use of induced mutants in recombination breeding. Mutants of no value often give promising recombinants when inter-crossed. Nichterlein (1999) enlisted a large number of new and better varieties of common bean (*Phaseolus vulgaris* L.) that were developed during 1960–1988 by using X-ray-induced bush type mutants in the pedigree. Mutation breeding programme for durum wheat in Italy involving extensive selection and hybridization work led to 11 registered varieties, five of which resulted from direct mutant selection and six from the cross-breeding procedures (Scarascia-Mugnozza et al. 1991). Mung bean variety NIAB Mung 98 was developed through hybridization between an induced mutant and an exotic accession (Siddique et al. 1999). Most of the mutant varieties of pulses, oilseeds, cereals and fibre crops released by Bhabha Atomic Research Centre, Mumbai in India are developed primarily by using mutants in cross-breeding.

2.9 Major Plant Traits Improved by Induced Mutations

2.9.1 Yield and Yield Components Improvement

Stable and high yield potential over a range of environmental conditions is probably the most important objective of most plant breeding programmes. One of the most important characters for judging agronomic value of mutants is their yield potential. Therefore, improvement in grain/seed yield has always been the main objective in almost all the crops. The improvement in grain yield through induced mutations has been brought about by alterations in yield contributing traits (Ahloowalia et al. 2004). Isolation of micromutations for higher yield coupled with some other desirable attributes like disease and pest resistance has been reported in chickpea (Kharkwal 2001, 2003; Kharkwal et al. 2001, 2005; Kharkwal and Shu 2009). Examples of important mutant cultivars with improved yield traits are listed in Table 2.2.

2.9.2 Tolerance/Resistance to Abiotic and Biotic Stresses

2.9.2.1 Tolerance to Abiotic Stresses

Abiotic stresses encompass several unfavourable environmental conditions such as drought, soil salinity, extreme pH, flooding and temperature. Induction of mutation for abiotic stresses has been successfully attempted in several crops and mutants for aluminium tolerance in banana (Matsumoto and Yamaguchi 1991), chlorate tolerance in barley and field pea (Kleinhofs et al. 1978), and salt stress in rice (Abe et al. 2007; Do et al. 2009), heat tolerance in rice (Poli 2013) and cold (harsh weather) tolerance in Amaranth (Gómez-Pando et al. 2009) and several other abiotic stress conditions have been obtained (Kharkwal and Shu 2009). Examples of important mutant cultivars with improved abiotic stress tolerance traits are listed in Table 2.3.

2.9.2.2 Tolerance/Resistance to Biotic Stress

Some of the crop varieties improved for yield or yield components through induced mutations have also shown improved tolerance to biotic and abiotic stresses. High yielding chickpea mutant varieties, Pusa 408, Pusa 413, Pusa 417 and Pusa 547 with resistance to Ascochyta blight, Fusarium wilt and other diseases and pests have been released for commercial cultivation in India (Kharkwal et al. 1988, 2005; Kharkwal and Shu 2009; Kharkwal 2017); similarly CM-72, CM-88, NIFA-95 and CM 1918 were released in Pakistan (Haq et al. 1988; Haq 2009). A number of mutants resistant to specific diseases or insect pests have also been isolated and released in several other crops. Examples of important mutant cultivars with improved biotic stress tolerance/resistance are listed in Table 2.4.

Table 2.2 Examples of mutants with improved yield traits

Crop	Latin name	Improved trait	Mutagen	Cultivar	Country	Reference
Rice	*Oryza sativa*	High yielding	Gamma rays	Zhefu—8	China	MBNL[a] Nos. 25 and 26, 1985
Bread wheat	*Triticum aestivum*	High yielding		Jauhar—78	Pakistan	Ahloowalia et al. (2004)
Barley	*Hordeum vulgare*	Semi-dwarf, malting quality	Gamma rays	Golden Promise	United Kingdom	Sigurbjornsson and Micke (1974)
Barley	*Hordeum vulgare*	Semi-dwarf	X-rays	Diamant	Czech Republic	Ahloowalia et al. (2004)
Groundnut	*Arachis hypogaea*	High yielding	Gamma ray	TAG-24	India	Ahloowalia et al. (2004)
Chickpea	*Cicer arietinum*	High yielding	Gamma rays	Pusa-547	India	Kharkwal et al. (2005)
Black gram	*Vigna mungo*	High yielding	Gamma ray	TAU-1	India	Ahloowalia et al. (2004)
Cotton	*Gossypium* sp.	High yielding	Gamma ray	NIAB-78	Pakistan	Ahloowalia et al. (2004)
Banana	*Musa* sp.	High yielding	Gamma ray	Al Beely	Sudan	PBGNL[b] Nos. 16 and 17, 2006

[a] *MBNL* Mutation Breeding Newsletter
[b] *PBGNL* Plant Breeding and Genetics Newsletter

Table 2.3 Examples of mutants with improved abiotic stress tolerance traits

Crop	Latin name	Improved trait	Mutagen	Cultivar	Country	Reference
Rice	*Oryza sativa*	Salt tolerance	Gamma rays	NIAB-IRRI-9	Pakistan	MBNL[a] No. 45, 2001
Rice	*Oryza sativa*	Salt tolerance	Gamma rays	VND95-20	Viet Nam	Do et al. (2009)
Rice	*Oryza sativa*	Tolerance to cold	Gamma rays	Kashmir Basmati	Pakistan	Ahloowalia et al. (2004)
Rice	*Oryza sativa*	Tolerance to heat	Gamma rays	Nagina-22	India	Poli (2013)
Rice	*Oryza sativa*	Salt tolerance	Ion beam irradiation		Japan	Abe et al. (2007)
Bread wheat	*Triticum aestivum*	Drought tolerance	Gamma rays	Njoro BW1	Kenya	IAEA Bulletin, 50-1
Barley	*Hordeum vulgare*	High altitude, harsh weather	Gamma rays	UNA La Molina	Peru	MBNL No. 43, 1997 Gómez-Pando et al. (2009)
Maize	*Zea mays*	Drought tolerance	Gamma rays	Kneja 698W	Bulgaria	PMR[b], 2012
Amaranth	*Amaranthus caudatus* L.	High altitude, harsh weather	Gamma rays	Centenario	Peru	Gómez-Pando et al. (2009)
Soybean	*Glycine max*	Tolerance to cold, drought and water logging	Gamma rays	Heinong-26	China	Khan and Tyagi (2013)

[a] *MBNL* Mutation Breeding Newsletter
[b] *PMR* Plant Mutation Report

Table 2.4 Examples of mutants with improved biotic stress tolerance/resistance traits

Crop	Latin name	Improved trait	Mutagen	Cultivar	Country	Reference
Rice	*Oryza sativa*	Resistance to blast and virus diseases	Gamma rays	Camago-8	Costa Rica	MBNL[a] Nos. 43, 1997
Bread wheat	*Triticum aestivum*	Resistance to black stem rust (Ug99)	Gamma rays	EldoNgano-1	Kenya	PBGNL Nos. 32 and 33, 2014
Barley	*Hordeum vulgare*	Powdery mildew	X-rays	Comtesse	Germany	MBNL No. 33, 1989 and No. 36, 1990
Barley	*Hordeum vulgare*	Mildew resistance	EMS	Betina	France	Sigurbjornsson and Micke (1974)
Chickpea	*Cicer arietinum*	Blight and wilt resistance	Gamma rays	Pusa-408, Pusa-413, Pusa-417	India	Kharkwal et al. (1988)
Chickpea	*Cicer arietinum*	Blight resistance	Gamma ray	Hassan-2K	Pakistan	Hassan et al. (2001)
Lentil	Lens	Blight resistance	Gamma ray	NIAB-Masoor	Pakistan	Sadiq et al. (2008)
Japanese pear	*Pyrus pyrifolia*	Black spot resistance	Gamma ray	Gold Nijisseiki	Japan	Saito (2016)
Peppermint	*Mentha piperita*	Wilt disease resistance	Neutron irradiation	Murray Mitcham	USA	Todd et al. (1977)

[a] *MBNL* Mutation Breeding Newsletter

2.9.3 Grain Quality and Nutrition

In recent years, there has been greater emphasis on the improvement of protein content and specific amino acids seed quality of cereals, pulses and oil content and fatty acid composition in oilseeds through mutation breeding. The alteration of seed colour and seed size for better acceptability has been achieved through induced mutations in several crops. The change of wheat seed coat colour from red to amber by gamma radiation resulting in the development of 'Sharbati Sonora' (Swaminathan et al. 1968) is a classical example. An increase in seed size and protein content associated with high yield in several chickpea mutant cultivars has been reported by Kharkwal (1998a). Chickpea variety Pusa 547 with large seed size, attractive colour and thin testa has been developed through radiation induced mutation (Kharkwal et al. 2005). Similarly, a high yielding early maturing chickpea mutant with high protein content, named as Hyprosola has been released in Bangladesh (Sheikh et al. 1982). Mutants with altered fatty acid composition have been isolated in soybean, rapeseed, sunflower, linseed and minor oil crops (Dribnenki et al. 1996). Examples of important mutant cultivars with improved quality traits are listed in Table 2.5.

2.9.4 Mutation Breeding for Improvement of Agronomic Traits

2.9.4.1 Plant Type, Growth Habit and Architecture

Dwarf and semi-dwarf mutants with reduced plant height having positive effect on yield via improved fertilizer response, increased tillering and lodging resistance was selected in cereals. In grain legumes, several examples of improved plant architecture, including dwarf or bushy mutants with increased lodging resistance, improved agronomic traits like plant architecture with erect growth habit and higher harvest index have been isolated (Kharkwal 1996, 1999, 2000) and released (Kharkwal et al. 2004, 2005; Kharkwal and Shu 2009). Semi-dwarfness and earliness are the characters most frequently described in released rice, wheat and barley mutant cultivars.

2.9.4.2 Flowering and Ripening Time

Changes in maturity period, leading to earliness, have been brought about by induced mutations in several crops. Rice variety IIT 60, an EMS-induced mutant of IR-8 matures 1 month earlier than IR-8 with same yield potential (Kharkwal 1996) and the improved rice variety Yuanfengzao, released in China, matures 45 days earlier than the original variety IR-8 (Wang 1991). In castor, the mutant variety 'Aruna' developed in India through neutron irradiation matures in 120 days compared to 270 days of the parent cultivar, HC-6 (Ankineedu et al. 1968). Several mutants showing extra-early maturity have been isolated and used in cross-breeding in various crops. Examples of important mutant cultivars with improved agronomic traits are listed in Table 2.6.

Table 2.5 Examples of mutants with improved quality traits

Crop	Latin name	Improved trait	Mutagen	Cultivar	Country	Reference
Rice	*Oryza sativa*	Grain quality	Gamma rays	VND95-20	Viet Nam	Do et al. (2009)
Rice	*Oryza sativa*	Grain quality	Gamma rays	ShweWartun	Myanmar	MBNL Nos. 11 and 12, 1978, Ahloowalia et al. (2004)
Rice	*Oryza sativa*	Glutinous endosperm	Gamma rays	RD-6	Thailand	Ahloowalia et al. (2004)
Bread wheat	*Triticum aestivum*	Grain colour	Gamma rays	Sharbati sonora	India	Swaminathan et al. (1968)
Bread wheat	*Triticum aestivum*	Grain colour	Gamma rays	Jauhar-78	Pakistan	MBNL No. 2, 1973
Sorghum	*Sorghum* sp.	Grain colour	Gamma rays	Djeman	Mali	MBNL No. 44, 1999
Chickpea	*Cicer arietinum*	Grain colour and cooking quality	Gamma rays	Pusa-547	India	Kharkwal et al. (2005)
Sunflower	*Helianthus annuus*	High oleic acid	Gamma rays	NuSun	USA	Ahloowalia et al. (2004)
Cassava	*Manihot esculentus*	Cooking quality	Gamma rays	Tekbankye	Ghana	MBNL No. 44, 1999
Grapefruit	*Citrus paradise*	Red fruit flesh and juice	Retrotransposon	Rio Star	USA	MBNL No. 37, 1991

Table 2.6 Examples of mutants with improved agronomic traits

Crop	Latin name	Improved trait	Mutagen	Cultivar	Country	Reference
Rice	*Oryza sativa*	Semi-dwarf	Gamma rays	Reimei	Japan	Das et al. (2017)
Rice	*Oryza sativa*	Short stature and semi-dwarf	Gamma rays	Calrose 76	USA	Rutger et al. (1977), Letsari et al. (2016)
Rice	*Oryza sativa*	Short stature, early maturity	Gamma rays	TNDB 100	Viet Nam	MBNL No. 45, 2001
Basmati Rice	*Oryza sativa*	Short stature and non-lodging	Gamma rays	CRM 2007-1	India	PMR No. 1 & 2, 2006
Bread wheat	*Triticum aestivum*	Short, resistant to lodging	X-rays	Creso	Italy	MBNL No. 6, 1973
Bread wheat	*Triticum aestivum*	Resistant to lodging	Gamma rays	Gergana	Bulgaria	MBNL No. 37, 1991
Barley	*Hordeum vulgare*	Semi-dwarf	X-rays	Diamante	Czech Republic	Ahloowalia et al. (2004)
Barley	*Hordeum vulgare*	Early flowering	Gamma rays	Mari	Sweden	Sigurbjörnsson (1975)
Castor	*Ricinus communis*	Early maturity	Gamma rays	Aruna	India	Ankineedu et al. (1968)
Rye	*Secale cereale*	Short lie cycle	Gamma rays	Soron	Peru	Gómez-Pando et al. (2009)
Banana	*Musa acuminata*	Short stature	Gamma rays	Novaria	Malaysia	MBNL No. 44, 1999; Mak et al. (1996)
Rice	*Oryza sativa*	Herbicide resistance	Gamma ray	Rice	USA	Maluszynski et al. (1995)
Rice	*Oryza sativa*	Herbicide resistance	Gamma ray	IRAT 239	Guyana	MBNL No. 33, 1981
Corn	*Zea mays*	Herbicide resistance	Gamma ray	Corn	USA	Mabbett (1992)

2.10 Social and Economic Impact of Mutation Breeding Technique in Crop Improvement

As the knowledge accumulated, it became clear that mutation technique was not a magic wand that would create anything at any time. Therefore, following mutagenic treatments, a mixed bag of induced variants is found and many of these may not be of any value. Because of these reasons, it will be unrealistic to expect miracles out of programmes using induced mutagenesis technique. Nevertheless, having already developed and released more than 3000 mutant varieties in a wide variety of crop species (Maluszynski et al. 2000), the accomplishments of induced mutagenesis technique in evolving superior crop varieties and its role in basic studies confer on it an honourable niche in the ever-growing crop improvement programmes and also contributing significantly in providing food security in several countries (Ahloowalia et al. 2004; Kharkwal and Shu 2009; Kharkwal 2017).

The following selected examples illustrate the role that mutation breeding has played and continues to play in plant breeding programmes for crop improvement. The rapeseed cultivar 'Regina II' was developed by mutation in Sweden and was released in Canada in 1953. 'Redwood 65' flax was derived and registered from a mutation programme at the University of Saskatchewan (Larter et al. 1965) and is present in the pedigrees of many western Canadian flax cultivars. Elsewhere in the world, almost 70% of the durum wheat in Italy was mutant varieties and there are 200 rice cultivars derived from mutagenesis programmes. The economic impact of mutant varieties of rice released with improved characters such as semi-dwarfness, earliness, improved grain yield, and disease tolerance and improved grain quality have been reviewed by Rutger (1992) and Maluszynski et al. (1995). Two improved rice mutant varieties, 'TNDB-100' and 'THDB', with earliness and improved grain yield were released within only 6 years after mutagenic treatment in Mekong Delta of Vietnam. These varieties grown in millions of hectares have maintained tolerance to acid sulphate soil or soil salinity (Do et al. 2009; Le et al. 2021). The economic impact of short straw mutants in barley that are in the pedigrees of many cultivars grown today derive from mutation programmes and have been of immense economic value in several countries. Michelite, an X-ray-induced white bean mutant with altered plant type, is in the pedigrees of most of the white beans grown in North America (Ahloowalia et al. 2004; Kharkwal and Shu 2009; Kharkwal 2017).

Mutation-breeding programmes in China for crop improvement was a profitable approach as the estimated output of cereals, fibre and oilseeds had substantially increased and contributed to national economy of China (Wang 1991). High yielding Chinese mutant varieties of wheat (Luyuan-502), rice (Ilyou-D069 and Zhefu-802), soybean (Hefeng-25), groundnut (Luhua-11) and other crops like cotton, maize and forage legumes are being cultivated in millions of hectares and their breeders have won National Invention Awards (Liu 2021).

Commercial success and economic impact of mutant varieties of grapefruit in Texas and pear in Japan has been highlighted by Nichterlein et al. (2000). Grapefruit mutant varieties 'Star Rubi' and 'Rio Red' are grown in almost 75% of the total grapefruit area in Texas, USA (Sauls 1999). The mutant variety 'Gold Nijiseeiki' of Japanese pear is more resistant to black spot disease caused by *Alternaria alternata* than its parent. The additional annual income by growing this variety is estimated to be about US$ 50 million (Amano 1997; Nakagawa 2021). In peppermint, the mutant variety, 'Todd's mitcham' forms bulk of the world's production of mint oil. Several high yielding and disease-resistant mutant varieties of chickpea, mung bean, wheat, rice and cotton released for commercial cultivation in Pakistan and grown in millions of hectares have made significant impact on the total production and productivity of these crops (Ahloowalia et al. 2004; Haq 2009; Kharkwal and Shu 2009; Kharkwal 2017).

2.11 Conclusion

Spontaneous mutations were the only source of genetic diversity available for the domestication and breeding of plants until the dawn of the twentieth century. It was nine decades ago that Stadler first reported induction of mutation in plants like barley and maize by X-rays to generate novel genetic diversity for crop improvement. The successful application of radiation for the induction of plant mutations prompted a flurry of research and development in its use to generate novel genetic diversity for crop improvement in several countries. This also prompted and lead to discovery of several new physical as well as chemical mutagenic agents. Since then, induced mutations have been used to generate genetic variation in many food, feed, ornamental and cash crops, leading to the release of large numbers of mutant varieties for cultivation across the globe. Global food and nutrition security in the coming years will also depend heavily on the availability and cultivation of improved crop varieties that can perform well under the pressures of increasing frequencies and intensities of drought, flooding and coastal salinity, warming temperatures and the transboundary spread of intensifying plant pests and diseases triggered by warming temperatures. Recent years are witness to the increasing application of newer mutagen sources and techniques such as High Hydrostatic Pressure, Ion Beams, Electron beams, Targeting Induced Local Lesions in Genomes, Cosmic rays for space mutagenesis and Endonucleolytic Mutation Analysis by Internal Labelling for the improvement of plant species, and of functional genomics technologies that establish gene-to-phenotype relationships for the discovery of molecular variants underlying mutations to be used in marker-assisted breeding and gene editing. The target of mutation breeding, therefore, will now get transferred from yield traits to quality traits and stress tolerance for adapting to climate change. The glorious history and outstanding achievements of mutation breeding during the past nine decades indicates that it is going to play a major role in shaping the future research on improvement of crop plants in twenty-first century and beyond.

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