Chapter

Potential of Mutation Breeding to Sustain Food Security

Arain Saima Mir, Meer Maria, Sajjad Muhammad and Sial Mahboob Ali

Abstract

Mutation is a sudden heritable change in the genetic material of living organism. Spontaneous mutation, the natural process that develops new allele copies of a gene was the only source of genetic diversity until the 20th century. Besides, mutations can also be induced artificially using physical or chemical mutagens. Chemical mutations received popularity due to its efficiency in creating gene mutations contrary to chromosomal changes. Mutation has played a vital role in the improvement of crop productivity and quality, resultantly > 3,000 varieties of 175 plant species have been developed either through direct or indirect induced mutation breeding approaches worldwide. The advances in plant breeding also achieved through molecular marker technology. The *in vitro* mutagenesis, heavy-ion beam, and space mutation breeding are being efficiently used to create genetic variability to improve various complicated traits in crop plants. In mutation breeding, TILLING (Targeting Induced Local Lesions in Genomes), a more advanced molecular technique is being used to identify specific sequential genomic changes in mutant plants. Therefore, the mutation breeding in combination with molecular techniques could be an efficient tool in plant breeding programs. This chapter will discuss and review the mutation breeding application for the improvement of crop productivity and environmental stresses.

Keywords: biotic and abiotic stresses, climatic changes, food security, ion beam, space mutagenesis, TILLING

1. Introduction

Mutation breeding also called “variation breeding,” is the procedure of exposing seeds to chemicals or radiation to produce mutants with desirable traits. The mutants created are called mutagenic plants or mutagenic seeds and can be used directly as a commercial cultivar or used as parent to breed new commercial cultivars. Although mutation breeding in the past fifty years was mainly focused on improving the yield specifically height reduction in wheat and rice, in contrast, today’s challenges are environmental stresses and its related effects and to motivating for climate-smart agriculture for food security [1]. In this chapter, the historical background of mutation breeding has been discussed chronologically. The types of mutants and mutations reported in worldwide literature are described. The natural and spontaneous mutations are elaborated with practical examples. All types of physical and chemical mutants and their success stories are discussed citing examples from all over the world. Lastly, the impact of mutation breeding on food security is explained with practical examples and achievements so far. The future prospectus of mutation breeding has also been discussed to highlight the significance of this important plant breeding process. This chapter provides a comprehensive understanding of the process with successful commercial examples of mutation breeding and the potential of this technique to meet future food security challenges. This chapter includes (i) introduction, (ii) historical background/development of mutation breeding (iii) mutation concept and its importance (iv) mutant crop varieties and their impact on food security (v) new breeding techniques (vi) prospects (vii)conclusions

2. Historical background/development of mutation breeding

The story of mutation and development of mutants in crop plants was first described in the book *Lula*, in 300 BC in China. The first natural mutant plant in cereals was found about 2317 years ago in China [2, 3]. Later, many aberrant plants with diverse variations were identified known as the first phase of mutation (1590-1868). The 2nd phase of the mutation was commenced in 1895 with the discovery of X-rays by W.K. Rontgen and the use of mutagens for the first time in 1897 to 1920 with the "Law of homologous series of variations" by N.I. Vavilov [2]. The chapter of mutation breeding was opened with the pioneered use of irradiation to create genetic variation by Lewis John Stadler in the 1920s.In almost the same time period Muller did his mutation experiments on fruit flies, Stadler was working on barley, maize and wheat manifested that radiation has power to create genetic variability in crop plants, although he was more interested in mutation breeding for fruit trees. Many geneticists believe the induction of mutation as a breakthrough in the history of genetics. American researchers were not so optimistic in their findings of the agricultural crops [3–5]. Chromosomal aberrations in Nicotiana were reported by Goodspeed and co-workers [6, 7]. The first-ever mutant variety "Vorsteland" of Tobacco with improved quality traits was released in Indonesia in 1934. Russian scientists, Delaunay and Sapehin reported the first wheat mutants with practical importance. German researchers started using mutation induction very early, but it was only theoretical in nature. The lecture on polymorphic factors in barley delivered by NILSSON-EHLE in Halle opened a new era in the use of induced mutation in Germany during the year 1939. In mid-thirties, he produced mutants at least one of those mutants [8] was equal to the mother genotype in yield performance. The experiments on induced mutation were extended after the cost for all work done till the 1940s was collectively funded by the A.B. Salts Jiiqvarn, Stockholm and Miirten Pehrsons Valsqvarn, Kristianstad, through Professor A. Akerman (head Swedish seed Association). This hard work led to the development of promising mutants in wheat, barley, oats, flax, soybeans, oleiferous and sweet lupine [8]. Stadler reported the production of solitary mutations and an increase in lethality by X-rays. Plants of different crop species respond differently to radiation doses. Cruciferae Seeds showed most insensitive sometimes tolerated 100.000r, whilst, the pea plants in contrast showed very sensitive with a maximum dose of only 10-20 r [9]. In the year 1942 and 1943, Eisleben Lien introduced comprehensive model experiments for barley. The use of induced mutation through radiation in crop breeding in Latin America was started during the 1960s initially in six countries viz., Colombia, Peru, Brazil, Guatemala, Costa Rica, and Mexico [10]. Rice breeding through use induced mutation started by china in 1960 and working continuously to improve the conventional and hybrid varieties. The first variety was the mutant developed in a series called 12 'Zhefu'. The most widely cultivated mutant variety between 1986 and 1994 was the Chinese variety'Zhefu802', which was evolved from Simei No. 2'. [10] Also reported radiation-induced biological effects in coffee breeding. In 1964 after the establishment of joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, mutation breeding was acknowledged as a greater tool by plant breeders globally [11]. Nuclear Institute of Agriculture (NIA) released its first mutant wheat variety Jauhar-78 in 1979 with salinity tolerance and shattering resistance and Kiran-95 released in 1996 endowed with better grain quality, tolerance to salinity and rusts. In 1977 Pakistan released its first rice mutant variety 'Kashmir Basmati from Basmati 370.Over 1000 mutant varieties in various crops have been developed by China in collaboration with IAEA and FAO in the past 6 decades. China started the use of space mutagenesis for crop improvement in 1987, Chinese scientists stated to produce giant sweet peppers and improved quality traits in wheat and rice through rare inheritable genetic mutations using space radiation (satellites and high-altitude balloons) [12]. Using space induced radiation, a number of advantageous mutations to make a breakthrough in most desired crop yield was also achieved [13–15]. The officially released mutant varieties in China accumulate around 741 of 45 crops and ornamental species [16, 17]. Recently, China has announced the launching of a new satellite for experiments in space on a variety of industries including agriculture [18].

3. Mutation concept and its importance

The word 'mutation' was coined by Hugo de Vries (1901) to represent a sudden heritable change occurring in the DNA of an organism caused artificially through irradiation, chemicals, viruses, transposons, or chromosomal aberrations that occur during reproductive processes [19]. These changes can be transferred to the offspring and are e differentiated in three general types namely gene mutation, chromosomal and genomic mutations. Induced mutation became the most frequently employed technique for developing novel improved germplasm in crop plants [20]. Mutation breeding is the application of mutagens to plant cells to accomplish crop breeding. Genetic variation makes the basis for the evolutionary process and breeding. In 1940, mutagenesis was adopted by the breeders as a tool that works faster to create mutations in plants [21]. Induced mutation breeding techniques have become most efficient, fast-tracking and widely exploited tools for crop improvement worldwide (**Figure 1**).

Mutation can be differentiated in three general types namely gene mutation, chromosomal and genomic mutations. However, mutation breeding is the application of mutagens to plant cells to accomplish crop breeding. Mutation provides the fundamental basis for a genetic variation on which genetic advancement and genetic drift depend and a single base mutation can cause devastating or beneficial consequences or no effect at all. Mutation breeding has played a significant role in crop breeding and genetics and genomic studies by generating a large amount of genetic diversity. Concurrently, climatic changes also threatening the food supply chain on the global level, resulting in fast loss of biodiversity for food and agriculture. The ongoing unpredictable climatic changes are the core problem in reducing crop yields worldwide, thus continuous development of new improved varieties for sustainable production is unavoidable. While the rate of natural mutations in the

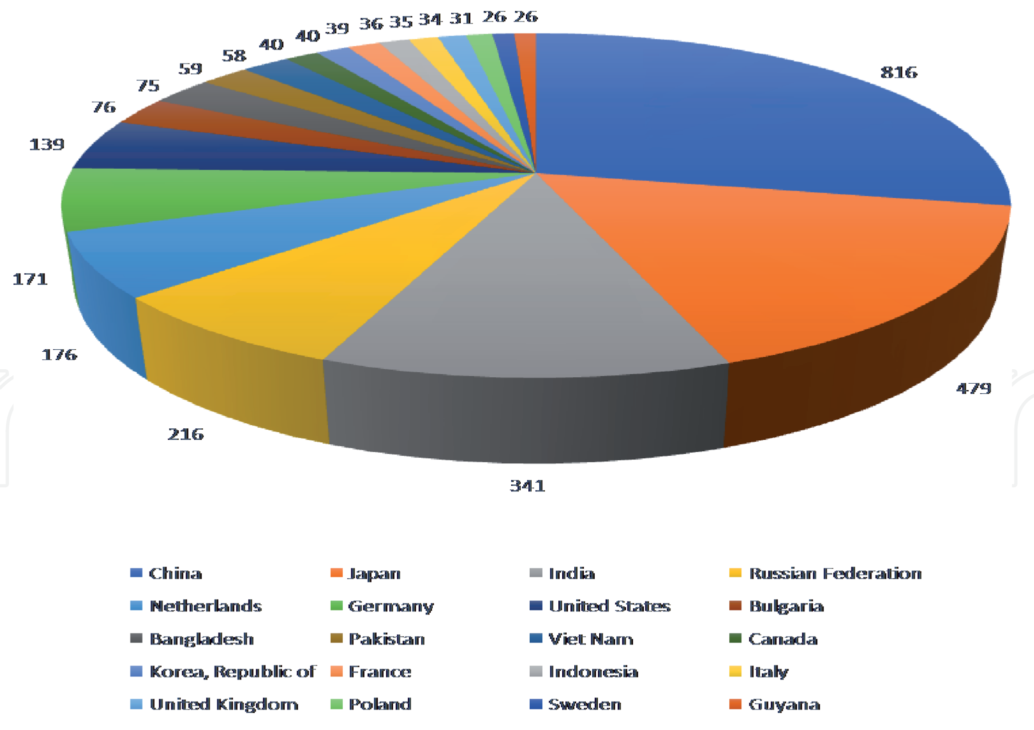

Figure 1.
Number of mutant varieties released in top 20 countries. Source: Mutant varieties database, IAEA accessed on 10th September, 2020.

crop plants is rare, thus use of induced mutation is indispensable to create genetic diversity for the desired traits for use in the breeding programs. Developing a new variety through mutation breeding reduces the time span for varietal development as compared to hybridization (**Figure 2**). Moreover, Mutants with multiple traits can be discriminated through mutation breeding, mutant varieties show a higher survival rate in the face of environmental swings. Mutagenesis is an efficient tool for generating mutations; these mutations can occur naturally or can be induced using mutagens, broadly classified as physical and chemical mutagens [22]. Mutagens offer more chances to acquire desired phenotypic changes and to study the genetic variations in relation to phenotypes and the annotation /deciphering of gene functions [23]. Various genetic resources of crop plants have been developed globally using different mutagenesis sources like EMS, gamma or X-rays and fast neutrons [24]. The crops like tomato have been focused after the availability of whole-genome sequencing data, which led to the identification of millions of single nucleotide polymorphisms (SNPs) and indels in tomato lines and in mutants [23]. In view of the introduction of high throughput next-generations equencing (NGS), several innovative approaches have been introduced for the discrimination of mutations in the mutagenized material. Some remarkable techniques are MutMap (mapping-by-sequencing) and MutChromSeq helpful to identify the basic changes induced through mutagenesis [25]. MutChromSeq helps to assort the desired genes in the shortest time span and has been successfully utilized in wheat and barley. Pakistan Atomic Energy Commission's (PAEC) first agriculture institute, Nuclear Institute of Agriculture (NIA)Tandojam" has exploited mutation breeding techniques since its inception in 1963 and developed 3 mutant varieties of wheat, 7 of rice, 1 of sugarcane, 5 of cotton, one each of lentil, mungbean and rapeseed through mutation breeding techniques. NIA released the first rice variety (Shadab) in 1978 from IR6 using ethyl methane sulphonate (EMS 0.5%) a chemical mutagen, variety had the potential to produce 7 tones/ha with superior grain quality [26]. However, the Nuclear Institute for Agriculture and Biology (NIAB) and other institutes of

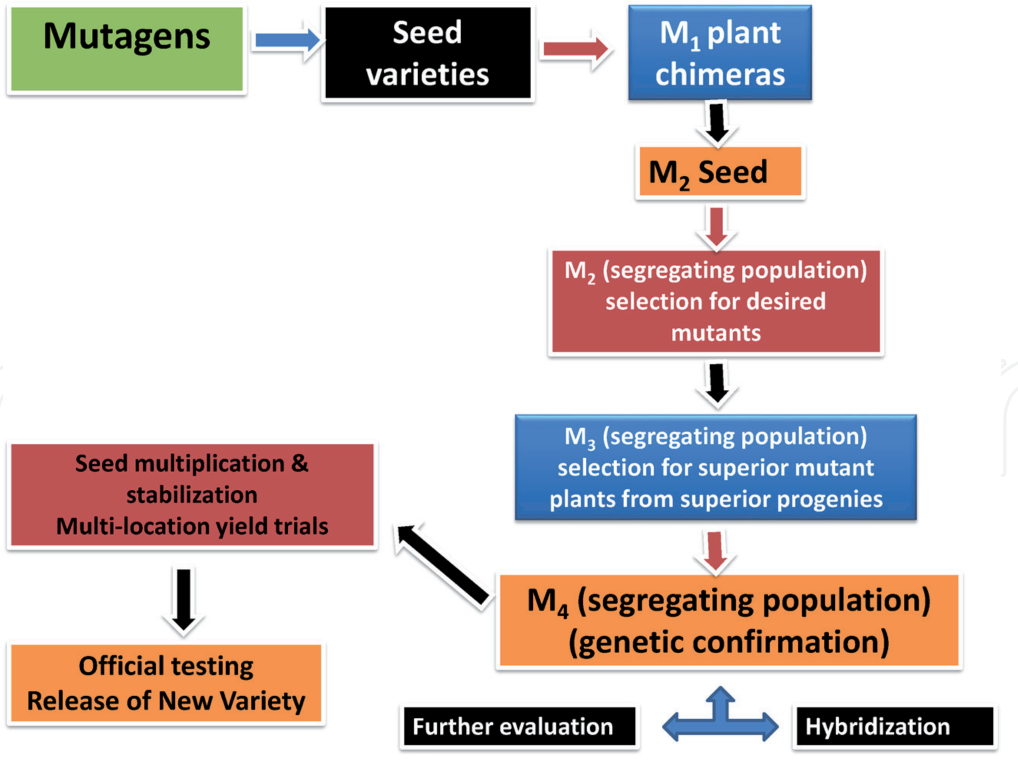

Figure 2.
Scheme of mutation breeding in crop plants.

PAEC have also developed mutant varieties of cotton, castor bean, sesame and mandarin thus helping the farming community by developing these improved varieties and boosting up their socio-economic status.

3.1 Spontaneous mutations

These are the genetic changes that occur due to chromosomal aberrations in the biological processes and serves as raw material for the evolutionary process. These mutations are the alleles of unknown genes which afterward given the name according to the phenotype or other related information viz., super-root (surl-7 to surl-7) [27], maize bronze (bz), carbohydrate accumulation mutant (caml) [28]. In maize spontaneous mutations occur in high frequency in the pollen part of some maize genotypes, but not in others [29]. Recessive mutations (one or two copies of the mutated allele produces the phenotype) are denoted by small letters, whilst dominant (one or two copies of the mutated allele produces the phenotype) and partially-dominant (one mutant allele produces an intermediate phenotype) are denoted by the first letter capital followed by the small letters. Most of the spontaneous mutations are point (single base pair change in the DNA) mutations. Gregor John Mendel was the first to quantitatively evaluate the dominance and recessiveness phenomenon in diploid organisms in 1866 [30].

3.2 Induced mutations

In addition to naturally occurring genetic mutations, novel alleles have been induced in plants by chemical and physical mutagenesis (**Figure 3**). The goal of mutagenesis is to induce genetic variation in cells that give rise to plants while minimizing chimeras, sterility and lethality [31]. Mutagenesis based breeding is primarily used to improve 1 to 2 main traits that effect on productivity or quality traits. More importantly is not under the regulatory restrictions faced by the genetically

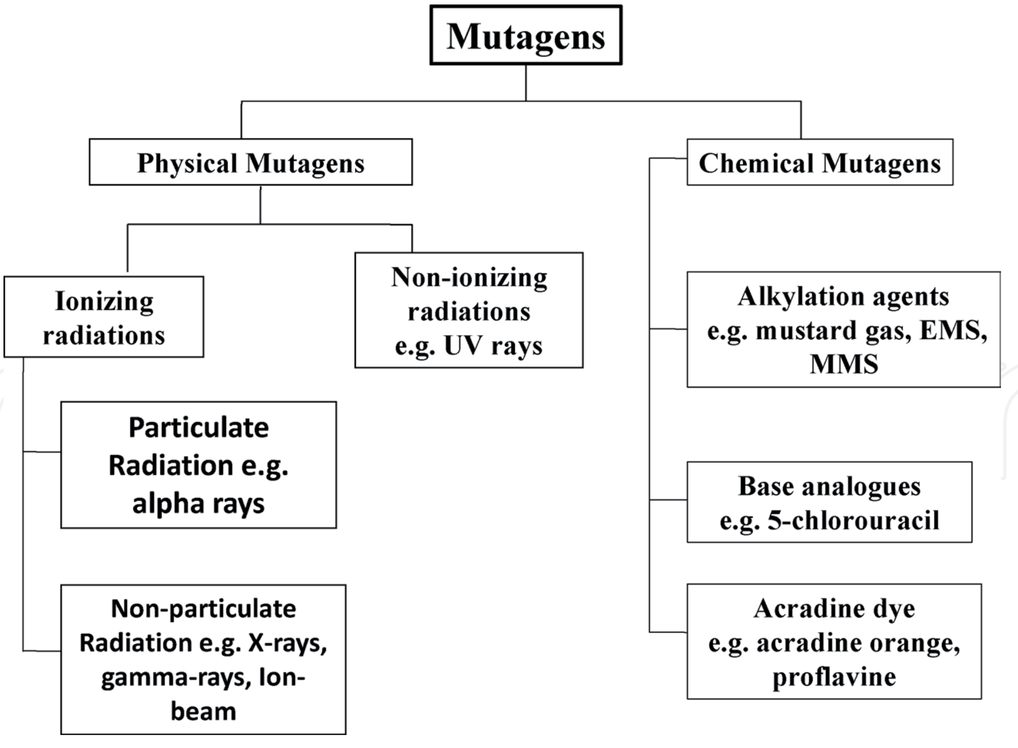

Figure 3.
Common mutagens used in plant mutation breeding. Source: Reproduced from FAO/IAEA, 2018.

modified organisms [32]. In some crops, chemically induced mutagenesis produced the desired phenotype in only several thousand lines. Today's high throughput phenotyping and next-generation sequencing methods have expedited the process to identify the mutants with desired genes (**Figure 4**). The use of engineered nucleases has helped to increase the accuracy of the mutation breeding through gene-specific mutation. Allelomorphic diversity induced in the gene of interest, whether spontaneously or experimentally, can be a great source for breeding programs to inculcate novel agricultural attributes [31]. Wanga et al. [33] used a combination of EMS and gamma radiation in sorghum but results were not recommendable. Although these are two major mutagens used to develop mutations [34, 35].

3.2.1 Physical mutagenesis

Physical mutagens namely X-rays, neutrons-alpha-beta particles, fast and thermal neutron, UV-light, especially gamma rays are used for the induction of mutation [36]. Physical mutagens are more common as compared to chemical mutagens (EMS) for mutagenesis. Physical mutagens like x-rays and gamma rays are preferred by the breeders as compared to the chemical ones. Gamma rays were used more frequently which accounted to improve 1604 mutants than the X-rays which improved 561 mutants [36]. Plant's exposure to X-rays provided the first ever undeniable evidence that phenotypic variability can be induced artificially. Hermann J. Muller was awarded Nobel Prize in 1946 in medicine/physiology for introducing irradiation using X-rays. Gamma-irradiation produces severe genetic mutations due to large chromosomal deletions and the re-enactment of the chromosome. Gamma rays have been used to induce mutations in seeds, cuttings, pollens and calli [37]. Since 1960 gamma irradiation has become the most popular and commonly used mutagens. This radiation-based mutagenesis was broadly used to improve mutant varieties directly as compared to other methods (acclimatization, selection, hybridization), comparatively, time-consuming, laborious and with

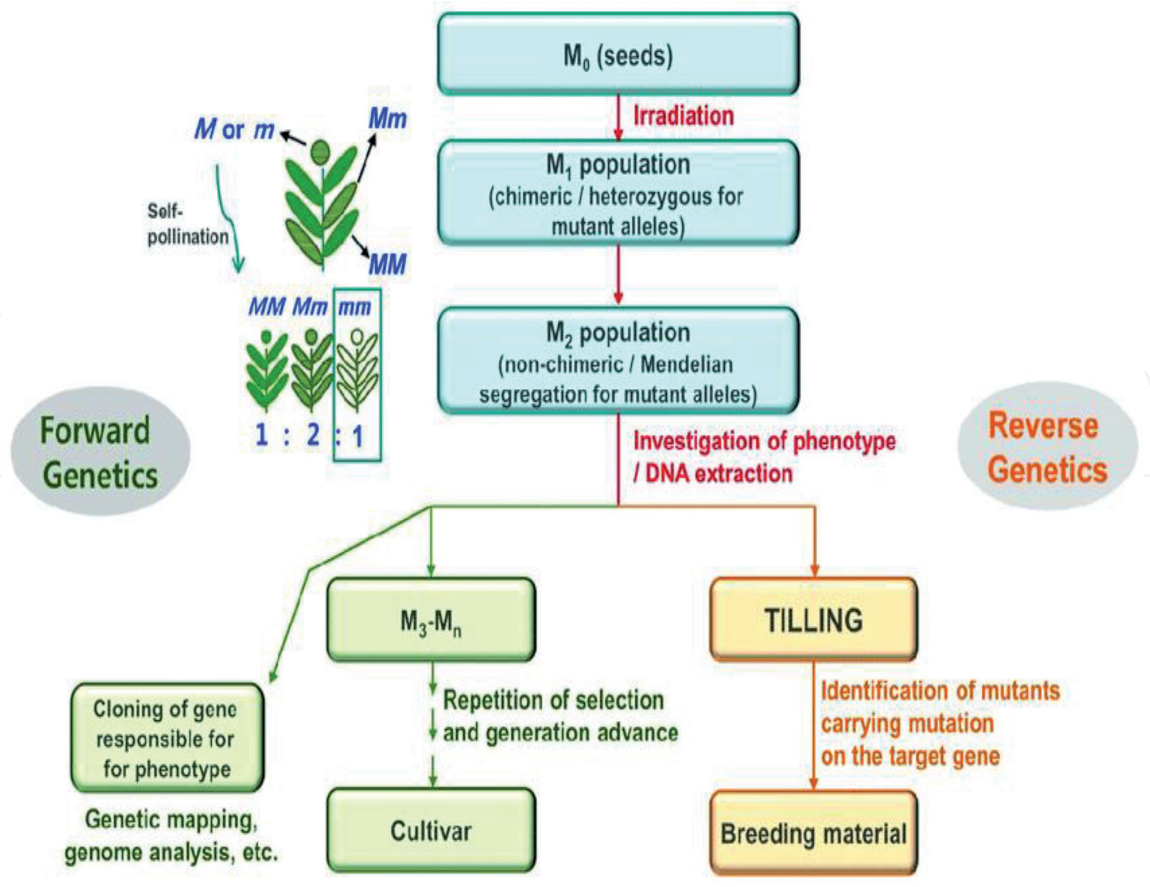

Figure 4.
Mutation breeding integrated use with modern techniques. Source: Directly taken from Jo and Kim, 2019.

lower genetic variation [38]. Fast neutron-induced mutagenesis is an exceptional technique among the other mutagenesis tools being employed in crop science in relation to higher impact. Fast neutrons normally cause deletions from a small number of bases to million bases [39]. Although, previously fast neutron was not as popular as other physical mutagens in plant mutagenesis [40].

3.2.2 Space mutagenesis

Space-induced mutation breeding uses cosmic rays to induce seeds in the space, for this experiment it is carried out in the satellites, space shuttles, and high altitude balloons and are considered beneficial over gamma radiation because of its lower damage to plants as compared to gamma rays on earth. Using space induced radiation, several advantageous mutations to make a breakthrough in yield were also achieved [13, 15, 41]. China has produced 41 varieties developed through space–induced mutation breeding of various crop species viz., rice, wheat, cotton, sesame, pepper, tomato, and alfalfa [42].

3.2.3 Ion beam mutagenesis

Heavy-ion bean is an important tool in mutation breeding since lower radiation doses are found to induce high mutation rates [43]. Due to its dense localized effect on DNA to effectively alter a single trait of the irradiated cultivar without damaging the rest of the characteristics, this technique is effectively being used in China and Japan to produce a large number of mutant varieties [44, 45]. In Japan, several ornamental plant varieties have been developed using high-energy ion beam irradiation while China is using low-energy Ion bean to create improved crop varieties. The initial plant varieties produced using Ion bean mutagenesis included carnation (*Dianthus caryophyllus*), Chrysanthemum (*Dendranthema Grandiflora*), and plants of Verbena sp. Afterward, several color and shape variations of petunia, Dahlia, and Torenia were also developed using this mutagenesis technique. Furthermore, the varieties developed using Ion beam mutagenesis include not only ornamental plants of high commercial demand [46], but also crops like salt-tolerant rice, citrus fruits, coniferous trees, mutant blast-resistant rice [47], mutant muskmelon, and rice varieties with lower fertilizer requirements [48].

3.3 Chemical mutagenesis

Chemical mutagenesis is the most efficient and expedient tool used for a large number of plant species. Ethyl methane sulfonate and sodium azide are the most widely used chemical mutagens to induce mutations in various crop plants like a tomato. The chemical mutagens used in mutation breeding are ethyl methanesulphonate (EMS), hydroxylamine, methyl methanesulphonate (MMS), sodium azide hydrogen fluoride (HF), and N-methyl-N-nitrosourea (MNU) [32]. Although, EMS is the most extensively used mutagen in plants due to its high efficiency at inducing point mutation (changes in a single nucleotide) and deletions (loss of chromosomal segment) in the chromosomal fragments. Mutant populations in various cereal crops using chemical mutagens for seeds or pollens have been developed comprising maize [49], barley [50, 51], rice [52], sorghum [53], and both hexaploid bread wheat [54] and durum wheat [55]. The EMS was exploited for potyvirus resistance in tomato [23].

4. Mutant crop varieties and their impact on food security

Mutation breeding techniques especially gamma and other physical mutagens have helped in generating a large number of mutants and generated a massive quantity of genetic variability that is significantly employed in the studies from plant breeding and genetics and in modern studies (genomics) (**Figure 4**). The mutants are released directly as varieties or furnish as a basic resource in the breeding programs to create genetic variation. The released mutant varieties offer higher yields, disease-resistant, improved quality, and resilient to environmental swings. A huge number of these mutant cultivars have been released in developing regions boosting up the economic status of these countries. These varieties are covering hundreds of millions of ha of agricultural land, whilst the impact on national economies of these countries is measured based on billions of dollars. The technique of mutation breeding is highly successful and its widespread implementation for crop improvement has led to the release of 3333 mutant varieties from 228 plant species (rice, wheat, and fruits like grapefruit, lettuce and others) in over 73 countries globally [56]. More than 1000 mutant varieties of major food crops covering millions of hectares, improving the rural economy, nutrition and helping in sustainable food security. Food insecurity is increasing worldwide and about 2 billion people especially in low and middle-income countries are undernourished. Concurrently, climatic changes also threatening the food supply chain on the global level, resulting in fast loss of biodiversity for food and agriculture. The ongoing unpredictable climatic changes are the core problem in reducing crop yields worldwide, thus continuous development of new improved varieties for sustainable production is unavoidable. While the rate of natural mutations in the crop plants is rare, thus use of induced mutation is indispensable to create genetic

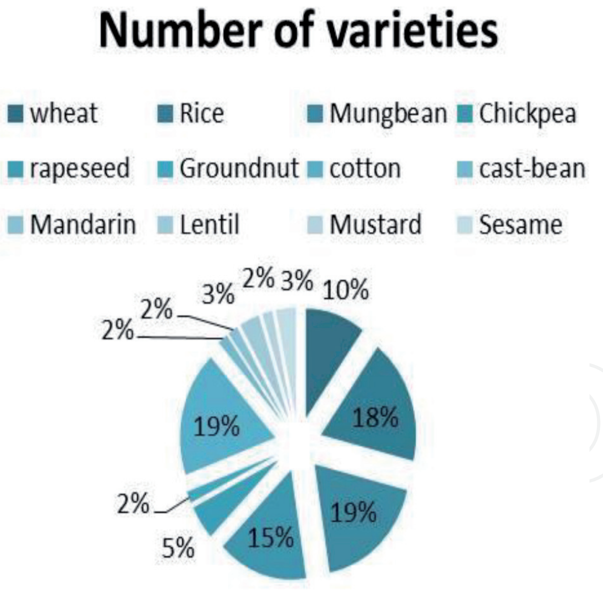

Figure 5.
Mutant varieties released in Pakistan. Data source: MVD/IAEA. Accessed on 13th September, 2020.

diversity for the desired traits for use in the breeding programs. The widespread use of mutation techniques in plant breeding programs throughout the world has led to the official release of more than 3200 mutant varieties from more than 200 different plant species, in more than 70 countries. In Pakistan, more than 59 varieties of different crop species (wheat, rice, cotton, sugarcane, mungbean, lentil, sesame, castor bean, mandarin, rapeseed, mustard, chickpea and groundnut) have been released through the use of mutation breeding (chemical and physical) techniques (**Figure 5**).

In China, only three mutant varieties are covering over 30 million ha and earn US$ 4.9 billion US dollars to uplift socio-economic status. In India, they have developed a huge number of mutant varieties and getting a large amount in return. In Bangladesh, Mutant rice varieties can be harvested a month earlier than the other varieties of rice-producing almost the same yield with superior quality. This variety is planted in three crop rotations and about 10,000 farmers cultivate this variety that is covering almost 80% of the area under rice cultivation. However, in Indonesia, an approximate amount of US$ 2 billion has been received from a single top rice variety. Many farmers' and millions of citizens getting benefits from the mutant varieties released by Indonesia. In Peru, improved barley and amaranth mutant varieties helping farmers to earn 7 million Andean and providing food and economic benefits thus improving their life status. In Vietnam, mutant varieties of rice and soybean helping poor farmers to improve their livelihood and a top rive mutant cultivar earning US$ 3.3 billion with an increase of US$ 537.6 million over old varieties. Whilst soybean mutant varieties bring about US$ 3 billion with 3.5 million farmers get a 30% increase in the economy. In Pakistan, 43 mutant varieties developed by NIAB showed an economic impact with earnings of US dollars 6 billion during 2018.

5. New breeding techniques (NBTs)

New breeding techniques or NBTs are a list of seven plant breeding techniques for incorporating genetic diversity into crop plants using site-specific targeted mutagenesis in the genome with greater accuracy and less off-targeted mutations [57]. The use of these NBT mutations is described as precision breeding. These techniques are zinc finger nuclease (ZFN) technology, oligonucleotide-directed mutagenesis (ODM), cisgenesis and intragenesis, grafting on GM-rootstock, RNA-dependent DNA methylation, agro-infiltration "sensustricto," and reverse breeding. The ZFN tool one of the site-directed nuclease (SDN) can be implemented to create a site-specific mutation in the plant genome. In addition, a number of new SDN techniques have been introduced viz. TALEN and CRISPR/Cas, and the latter is now extensively being used [57]. Recently, IAEA and FAO jointly launched a program known as Plant Mutation Breeding Network (PMBN) on the basis of a large number of crop varieties (2000) in the Asia Pacific region [58]. Out of these, 826 rice varieties to date have been released using mutation breeding, of these 699 were from the Asia-pacific region, with 290 from china. This program will be beneficial to farmers and researchers by developing new improved varieties with a higher yield, stability, and quality traits, disease resistance and resilience to changing climates through mutagenesis. The PMBN will work to further expand these great achievements jointly among the member countries. The conjoint use of classical mutation breeding method through screening of TILLING populations NBT mutations can be employed implicitly in the modification of plant attributes. The main advantage of NBT over the classical mutation technique is its precision and specificity that could be utilized to find robust mutation sites without the unwanted genetic changes that are the main problem in the classical mutation breeding. Resultantly, desired mutations could be retrieved through traditional mutation techniques. This is a lengthy process but of high applicability because of efficient tools to create mutant populations and to screen these mutations for targeted genes [59].

6. Prospects

With the rising food demands, the development of new crop varieties with improved yield potential and better resistance to biotic and abiotic stresses is vital. Modern techniques, molecular, and omics are the tools in hand to speed up the breeding route in integration with conventional (mutation/hybridization) methods. The integrated approach of using genomic and omics data with genetic and phenotypic data helps to unfold the genes/pathways connected with desired traits [60]. The conventional breeding methods have been employed extensively in combination with transformation, gene editing and marker-assisted selection (MAS). The selection of suitable parental materials endowed with desired traits in different crop species is fundamental for any successful breeding program. The highly favored markers known as Single Nucleotide Polymorphisms (SNPs) are helpful to analyze genetic variability and population configuration, in constructing genetic maps and to present genotypes for GWAS (genome-wide association analysis) [61]. Single-nucleotide polymorphisms (SNPs) are markers of choice to detect genetic diversity in crop plants [62]. Genotyping by sequencing (GBS) technique is based on next-generation-sequencing also done with SNP markers to incorporate high throughput genotyping [63]. These molecular techniques in combination with NBTs can do a miraculous job in the future to develop environment resilient cultivars to help fighting food security.

7. Conclusions

Mutation breeding has substantially contributed to crop improvements worldwide. Thousands of mutant crop varieties released in different countries have significantly improved yield potential, nutritional quality, biotic and abiotic stress tolerance. Several mutants with one or few desirable traits in different crops or vegetables are widely used as parents for breeding new commercial cultivars. Besides developing thousands of crop varieties, mutation breeding has created tremendous genetic resources for all major crops and vegetables worldwide. The integration of the latest mutation breeding tools with robust selection and speed breeding tools increases its scope in meeting food security challenges with exponentially increasing human population and climate change scenarios.

Conflict of interest

The authors declare no conflict of interest.

Author details

Arain Saima Mir[1*], Meer Maria[2], Sajjad Muhammad[2] and Sial Mahboob Ali[1]

1 Nuclear Institute of Agriculture (NIA), Tando Jam, PO Box 70060, Pakistan

2 Department of Biosciences, COMSATS University Islamabad (CUI), Park Road, Islamabad, Pakistan

*Address all correspondence to: saimamir_nia@yahoo.com

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