Chapter 4

Induced Mutation: Creating Genetic Diversity in Plants

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Additional information is available at the end of the chapter

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Abstract

Genetic diversity is the variation occurred in genetic information, which depends on frequency and diversity of alleles among individuals within a population or a species. This phenomenon, which is also a part of the evolution process, allows the organisms to adapt to changing conditions and to survive. Populations with high allelic variability are more easily adaptable to changing environmental conditions. However, nowadays, constant use of populations with certain characters in the plant breeding and the uniformity of consumer demands are among one of the causes of genetic erosion. Loss of genetic diversity within a species can lead to loss of useful properties for human beings. If stress conditions such as disease or drought occur, the ability of a population to survive by adapting to this new condition is dependent on the presence of individuals carrying gene alleles that need to adapt to these conditions.

Keywords: gamma rays, genetic diversity, induced mutation, plant

1. Introduction

The first information on the importance of genetic diversity begins with Darwin, which emphasizes the importance of variations in the process of adaptation to natural habitat. In the process of surviving species that can adapt to changing environmental conditions and the disappearance of others, the factors that caused them were researched and the definition of alleles was first used by Mendel, who is considered the father of heredity. Alleles do not only create the source of similarities and differences between progenies, but also makes it possible for species with high genetic diversity to continue their evolutionary process by adapting to different conditions. A biochemical approach to genetic diversity was presented by the introduction of isoenzyme techniques in the mid-1960s. In the following years, the discovery of the structure of DNA and its DNA-based examination of diversity has enabled scientists to reach

accurate information about genetic diversity, gene flow, and the origins of species. According to the "Neutral Theory of Molecular Evolution" first introduced by Motoo Kimura in 1968, many of the evolutionary changes arise from the genetic drift of the neutral mutant alleles. Greater than 100 bp insertion/deletion is rapidly removed by natural selection that has caused a great change and damage to the DNA. According to the neutral hypothesis, mutations that occur with smaller changes are harmless mutations. They are protected in the evolutionary process by contributing to the formation of genetic diversity. Most of the polymorphisms are kept in a population by mutation and random genetic drift [1–7].

The genetic difference between individual of a species is the basis for evolution and adaptation. If all of the individuals were genetically identical, species could not be survived under changing environmental conditions such as drought and salinity. It is known that there is a positive correlation between fertility and viability with population size and genetic variation. For this reason, regardless of whether the existing genetic diversity is beneficial, it should be considered that it poses a potential against rapidly changing environmental conditions [8, 9]. Loss of genetic diversity will adversely affect the ability to deal with environmental change in the evolutionary process. It is anticipated that, particularly small and isolated populations will be more affected by this loss [4]. Although loss of genetic diversity has been thought to be a threat to rare species for a long time, it has also been found to be effective on widespread species with large populations [4, 10, 11]. Furthermore, the fertility and viability rate of populations with reduced genetic variability and loss of valuable alleles, especially as a result of self-destruction, is diminishing. Random genetic drift changes the frequency of alleles between generations. The frequency of some of the alleles may decrease while the other allele may become more common. Naturally occurring mutations also support this process. The genetic variation among individuals is the basis for the evolutionary change of species, populations, and progeny. Evolutionary change requires modifications of genes or gene combinations. The functions of the existing alleles are altered by mutation or recombination. Appropriate mutations can help the organism better use the current environment. Alleles that are previously neutral or have little effect on the reproduction success of individuals may suddenly become important. Obtaining useful genetic variations through natural mutations is a very slow process. In the future, it is impossible to predict which alleles will be necessary for a survival. For this reason, populations and species with high genetic variation are more likely to have alleles that may be necessary for adaptation to changing conditions. Generally, natural populations have the variation to be expressed in the case of the change of selection pressure. The basis of plant breeding is also based on this genetic variation. By artificially altering the selection pressure, some alleles come forward and populations gain new features. Since there is a positive correlation between population size and genetic diversity [12], decreasing population size or limiting gene flow between subpopulations may cause a reduction in genetic diversity [13]. Genetic drift with a positive role in the evolutionary process [3], inbreeding, mating between close relative individuals, reduction of viability and fertility of individuals in the population may cause effects such as the reduction of heterozygote, allelic losses, and some alleles become fixed in the population. As a result, genetic variation will be lost in populations [9, 12, 14]. Many populations are so small that genetic drift, a random process, has an important influence on the number of alleles that will be transferred to future generations. The useful

alleles transferred from previous generations may be lost as a result of genetic drift [1]. Two main consequences of genetic drift; (a) different alleles frequencies between generations are irregular and (b) the genetic diversity of the populations is lost. At the beginning, rare alleles will disappear and the mean heterozygote will decrease over time. While genetic diversity within populations decrease, genetic differences between populations may increase or decrease depending on whether the random genetic drift in different populations in the same or opposite direction. This loss will continue until the gene pool is stabilized for an allele or until a balance is established between loss of genetic variation and genetic variation through mutations. The loss of both rare alleles and heterozygotes, which cause the decrease in genetic diversity in the population, will decrease in an inverse proportion to the effective population size (Ne). The population size (Ne), which helps to determine the demographic structure of the population, makes it possible to predict the change in genetic diversity. Population size is an important factor that increases the genetic diversity of individuals' intra-species and between taxa. Besides genetic diversity of non-endangered species is higher than endanger species [6, 15–17]. Only a small amount of gene flow may be sufficient to prevent the loss of genetic diversity in a population. Especially, the accumulation of mutations with small deleterious effects in the alleles may reduce the viability and fertility of the populations. These mutations never reach high frequencies in large populations of sexual reproduction. The selection pressure prevents these harmful alleles to become widespread. However, in isolated small populations, the frequency of these harmful alleles may increase and be fixed by chance, if genetic drift may be stronger than natural selection. When a harmful mutation is stabilized in the genetic construct, it causes the population size to decrease and other harmful mutations to be fixed [18]. While the destructive effect of detrimental mutations affect large populations, thousands of years later, can be seen in minutes in small populations. This event, known as mutation meltdown, reduces the size of the population depending on the accumulation of detrimental mutations. The mutation meltdown, is a genetic problem, especially for small populations of endangered species, occur depending on mutation characteristics, demographic characteristics of the mutation and population, and the relationship between mutations and their adaptation [19, 20]. Species or populations suffering from the genetic bottleneck disappear over time. Adaptation can sometimes be a rapid process involving a single gene. In cases when the positive selection is strong, the best allele is fixed in the relevant locus and the adaptation process is terminated unless a better allele is generated by mutation or a new allele is introduced into the population by gene flow. This does not occur in polygenic characters. Genetic drift has a significant impact on the protection of genetic diversity and the amount of genetic variation among populations. Genetic drift as well as mutations, selection, and gene flow are factors contributing to genetic diversity. Sometimes, however, subspecies can occur as a result of gene flow, and which can adversely affect fertility or cause outbreeding depression [9, 21].

2. Natural mutagenesis

When damage occurs in DNA due to a physical, chemical, or biological agent, molecular systems recognize and repair this damage. When this mechanism is unsuccessful, mutation

occurs in the organism [22]. Mutagenesis, a consequence of errors in DNA repair, is a mutation-producing process. Hereditary changes that occur naturally and suddenly, which are not caused by recombination and segregation, are called mutations [23]. Mutations that lead to the formation of new individuals, species, and genera are considered as the most important factors of evolution since they can be transferred to future generations [24]. Mutations that occur in somatic cells are not transferred to future generations, but they are important for vegetative produced species. Mutation-derived individuals are called "mutants" [23]. Natural mutants formed in the evolutionary process are one of the most important factors contributing to the formation of species. The rate of spontaneous mutations in higher plants is quite low (10^{-5}–10^{-8}) [25]. Mutations occur more frequently in some regions of the genome. For example, in almost all organisms, the mutation rate in GC regions is higher than in AT regions [26]. While deleterious or neutral mutations that form a part of the mutations that occur in the natural process may disappear in the evolutionary process, the protected mutants may have desirable agricultural characteristics or easily adapt to changing environmental conditions [25]. The first document relating to mutant selection belongs to the year 300 BC. The description of various wild and cultivated mutants was made by Linnaeus in the second half of the 1700s [23]. Although hereditary variations have been observed and used for thousands of years, the mechanism of heredity was first revealed by Mendel [27]. Johanssen's research on seed index of common beans in 1913 can be considered as the first to prove the presence of natural mutations with small effects. In 1924, Baur emphasized that the accumulation of these small mutations in the genome over the years had an impact on the evolution process [28]. Mutation term was first used by de Vries at the beginning of the 1900s. The first evidence of mutation breeding work, which will gain a new perspective on plant breeding, was obtained in 1927 in *Datura stramonium* by radium ray application [20]. The process that begins with human being's awareness of natural mutation has led to the development of many new varieties with induced mutation.

Mutations can occur in spontaneously or under different influences of various mutagens. For this reason, there are various classifications made by different researchers [2, 29–33]. Yüce et al. [34] classified mutations into two main categories as genomic and plasmon mutations. Genome mutations; (1) "gene mutations" resulting from genetic changes, (2) "Chromosomal mutations" formed by chromosome aberrations or chromosomal changes, (3) "Ploidy mutations" resulting from genome and chromosome number changes, (4) Mutations created by transposition elements, and (5) Mutations resulting from somaclonal and gametoclonal variations were classified as five subgroups. Mutations occurring in the genetic material of mitochondria and plastids in the cytoplasm are classified as "plasmon mutations" in a single heading [34].

Gene mutations are structural gene changes in DNA that occur through different mechanisms such as deletion, insertion, and substitution. Intercalar substances, ultraviolet rays, alkylating compounds, and free radicals cause gene mutations [33, 34].

Chromosome mutations are genetic changes that are generally caused by deletion, duplication, inversion, and translocation mechanisms and are larger than gene mutations [33, 34].

Ploidy mutations are divided into two main groups as polyploidy and aneuploidy. The smallest ploidy level is the haploid in the gametes, containing n chromosomes. Eukaryotes contain diploid (2n) chromosomes in cell nuclei. Cells that contain more than two genomes in

the nucleus are called polyploids. Polyploidy are very common in plant kingdom and are naturally seen in important cultivated plants such as wheat, cotton, potato, banana, and coffee. These species, which contain more than two alleles in terms of genetic structure, display a richer genetic variability. Aneuploidy is the number of chromosome changes that occur as an increase or decrease in the number of chromosomes. In this case, individuals with fewer or more chromosomes than normal chromosomes are formed [34, 35].

Transposon elements are the mobile genetic elements found in the genome and cause mutation due to their ability to displace within the genome [36, 37].

Somaclonal and gametoclonal variants, another source of mutations, are genotypic or phenotypic differentiations in somatic or gametic cells, which are formed by hormones used in tissue culture media [34, 38, 39].

Spontaneous mutations caused by disruptions in the functioning of molecular mechanism in the cell, the main source of genetic diversity [40]. In every generation, 10^{-5}–10^{-6} mutation rate per gamet cell occurs. This ratio can vary between genes and even by regions within the genes [41]. Although mutations occur infrequently, when considered as a whole genome, it plays an important role in the change of genetic diversity. Because mutations occur at randomly, and it cannot know which one of the gene copies will mutate in diploid or polyploid organisms [22].

Spontaneous mutations have been the basis for the beginning of agriculture and for humankind to pass on a settled life. Self-changing hereditary features have made the dormancy period reduced in species such as peas, wheat, and barley. In addition, the loss of bitterness was formed in almond, linden, watermelon, potato, eggplant, cabbage, and various hazelnut species. All these developments have made these products suitable for human consumption. Another spontaneous mutation was the formation of parthenocarpy in grape and banana (**Table 1**). Naturally occurring mutations have led humans to work on induced mutations [42].

Mutation that facilitated domestication	Examples of plant
Abolishment of bitterness and toxicity	almonds, lima beans, watermelons, potatoes, egg-plants, cabbages nuts
Abolishment of the need for sexual reproduction (seedlessness or parthenocarpy)	bananas, grapes, oranges, and pineapples
Loss of natural seed dispersal mechanism—shattering of pods and heads	peas, wheat, barley
Loss of the hard seed coat and other germination inhibitors (dormancy)	wheat, barley, peas
Facility for self-compatible hermaphroditism	grapes, papaya, *etc.*

Table 1. Useful properties acquired by spontaneous mutations in evolutionary processes in plants (Table was directly taken from Mba [42]).

3. Induced mutation

When it considered the changing environmental conditions and population growth, it is necessary to increase agricultural products approximately 70% in near future [43]. However, breeding trials for the development of desirable agricultural characteristics cause genetic bottleneck. Genetic resource erosion in plants will also lead to the loss of useful genes that would potentially create for breeding studies [44]. Induced mutations may help regain lost traits due to reasons, such as stress factors in the evolutionary process. These genotypes, exempted by the ethical and legal limitations faced of genetically modified products, can be identified by advanced molecular techniques. Thus, the variation of the mutants with the new phenotypes revealed can provide a different perspective for plant breeding studies [45]. Mba et al. [46], referring to the importance of landrace and wild varieties as important genetic resources in breeding strategies, proposed that artificial mutation of putative parental materials in order to create new alleles controlling the desired characters for the twenty-first century "smart" crop varieties [42].

There is a 125-year history of studies on induced mutation. It was determined that X-ray, alpha, beta, and gamma rays are the source of radiation, with different studies in 1895–1900. In 1897–1908, the first studies were carried out to investigate the effects of radiation on plants in 1901 and 1911, it is proved that mutation was induced by chemicals at the first time. In 1904 and 1905, Hugo de Vries suggested that radiation promoted artificial mutation. In 1910, Thomas Hunt Morgan did his first mutation experiments with *Drosophila melanogaster*. In 1927, Muller proved precisely that the X-rays induced mutation. In 1928, Lewis John Stadler successfully induced mutation in corn and barley using X-rays. In the years 1934–1938, Tollenar improved the first commercial variety of tobacco called "Chlorina," and this variety was released in Indonesia [28]. After these initial developments, the curiosity and research of the scientists against the induced mutation have continued.

Today, there are 3222 commercial mutant varieties according to the IAEA data. The countries where the most mutant species are released are China (810), Japan (481), and India (330). According to this data, the highest mutant cultivation rate is in Asia continent [47]. When the products are examined, the percentage of mutant varieties by mutation breeding are constituted of 49.5% cereal, 21.9% ornamental plants and flowers, 15% legume, 2.4% fruit nuts, 2.4% vegetable crops, 2.3% fiber crop, 2.1% oil crops, 1.2% forage crops, 0.6% root-tuber crops, 0.4% herbs, 0.2% medicinal plants, and 2% other crops [48].

Mutations can be induced by biological, chemical, or physical factors as well as spontaneous [40]. In breeding studies, physical and chemical mutagens are generally preferred, but mutations also can be generated by biological agents such as viruses and bacteria. While X-rays, γ-rays, fast neutrons, ultraviolet (UV) rays, beta particles, alpha particles, protons, and ion beams are used as physical mutagens, as chemical mutagens; alkylating agents, azide, hydroxylamine, antibiotics, nitroso compounds, acridines, and base analogs are used for mutagenesis [32, 47]. However, "insertional mutagenesis" and "site-directed mutagenesis" methods, which give more precise results in parallel with progress in genome and sequencing studies, are predicted to be used more widely in future mutation breeding studies [49, 50]. Mutant plants

were mostly developed using physical mutagens. Physical mutagens are preferred to chemical mutagens for reasons such as ease of use, low cost, and nontoxicity. In particular, gamma and X-rays are the most commonly used mutagens. About 64% of the mutant plants obtained with the physical mutagens were improved using gamma rays [51–53].

Different plant parts such as seed, meristem, callus, and anther can be used in induced mutation studies. Mutation studies begin with the initial phase, called M_0, where different plant materials are used. Each generation of the mutation continues in the form of M_1, M_2, M_3,…. When a seed is used as the starting material, homohistont generation is obtained at the M_2 stage, while the number of cycles may increase in mutagenesis using vegetative tissues. Screening and selection starts with the first homohistont generations. Once these stages are completed, experiments are carried out to release the mutants obtained, or they can be used as parents in breeding programs [23].

4. Use of induced mutations in the enrichment of plant genetic resources

Induced mutation studies were initially conducted only in field conditions. Tissue culture studies, began with cell culture at the beginning of the twentieth century, have become widespread parallel to the development of technology and have enabled the rapid and disease free reproduction of many plant species. *In vitro* mutation may be preferred for tolerance selection, especially for stress and diseases, because of the shortening of the selection period, being economical and the need for small areas in the mutation studies. Generally, plants are transferred to field conditions after the selection made in step M_1V_3 *in vitro* and in this way provides the researcher time and labor savings.

In vitro and *in vivo* conditions, different plant explants and different doses of the mutagen to be administered have used to create variations in plant species. High dose applications will increase mutation frequency in induced mutation studies to create genetic diversity in plants. In this case, however, the percentage of survival of the plants will either be too low or the plant will die [54, 55]. For this reason, appropriate dose determination is required for each mutagen and plant species. Appropriate dose determinations are made on the basis of M_1 plants survival rate and vegetative growth, primarily shoot height [56]. A sample graph, appropriate doses calculated according to survival rate of some common bean cultivars by gamma irradiation are given in **Figure 1** [57].

Numerous studies have been carried out for enriching genetic variation by plant-induced mutation and using this variation to the benefit of humankind (**Figure 2**). Some of these studies were conducted in order to create polymorphism by removing genetic bottleneck. In this way, new hybrid groups can be formed. Genetic diversity is also important for breeding programs and the sustainable use of genetic resources [58]. Genetic variation obtained from induced mutation has contributed to modern plant breeding. Studies conducted by mutation breeding can be summarized under the titles of biotic and abiotic stress tolerance, improving plant nutritional properties, and increasing polymorphism. Barakat and El-Sammak [59] in *Gypsophila paniculata* L., Kaul et al. [60] and Barakat et al. [61] in chrysanthemum obtained mutant

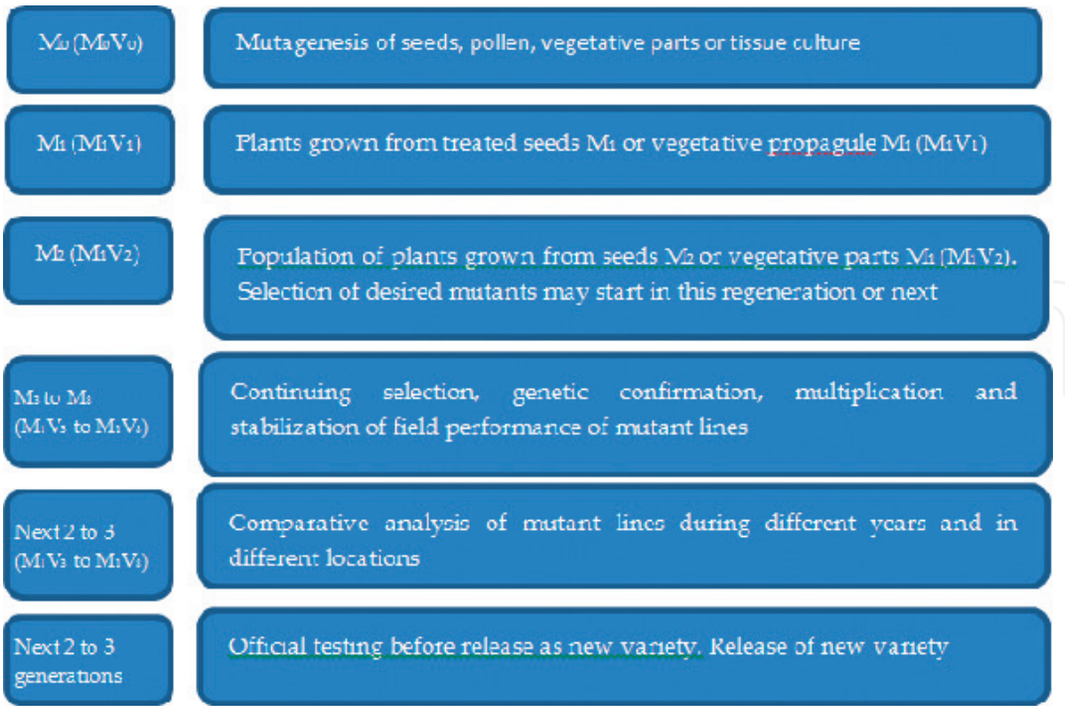

Figure 1. Stages of mutation breeding (Figure was directly taken from Jankowicz-Cieslak et al. [27]).

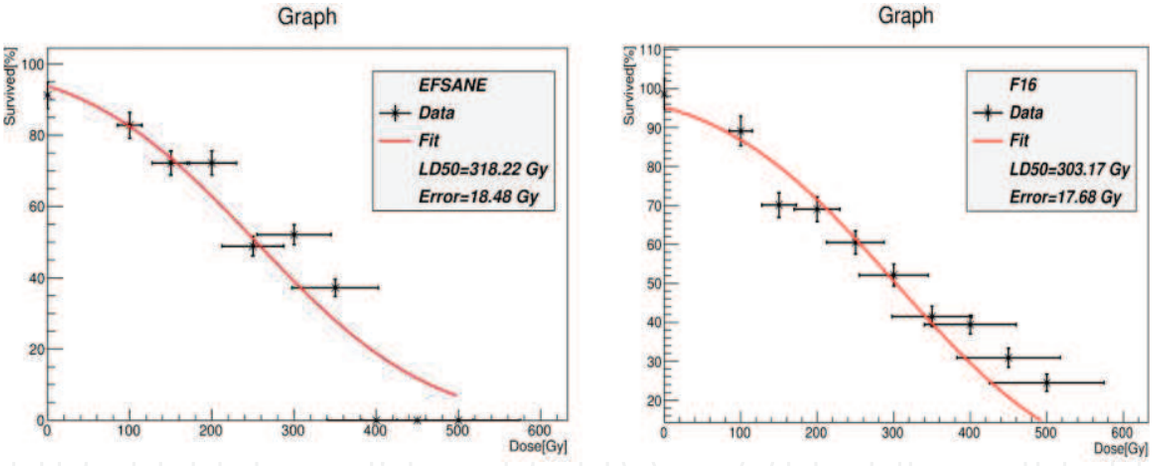

Figure 2. LD50 values of two common bean cultivars (figure was directly taken from Ulukapi and Ozmen [57]).

plants and they identified mutants' genetic similarity with molecular markers (from 0.59 to 0.97, 0.06 to 0.79, and 0.43 to 0.95, respectively). About 83% polymorphism was detected in the chrysanthemum as a result of gamma-induced mutation. It has been reported that the 30 Gy gamma dose is the most effective dose for in vitro genetic variation [62]. Wu et al. [63] have developed resistance at varying frequencies to blast, bacterial blight, and tungoric disease using both chemical (diepoxybutane and ethylmethanesulfonate) and physical (fast neutron and gamma ray) mutagens in rice. The semidwarf rice mutant "Calrose 76" released in California and the short height mutant rice called "Basmati 370" in Pakistan were improved

[64]. EMS is the most preferred chemical mutagen. It causes a single nucleotide polymorphism (SNP). In this way, even a single change in the genomic coding sequences will change the expression of the gene, causing changes in transcription and translation products [65]. Till et al. [66] developed two mutant rice populations using ethyl methanesulphonate (EMS) and a combination of sodium azide plus methyl-nitrosourea (Az-MNU). The investigators have screened target genes and identified 30 nucleotides changes in Az-MNU population and 27 nucleotides in the EMS population. In a study using EMS as a chemical mutagen, four populations with different mutation densities were developed on soybean. The results are described by Targeting Induced Local Lesions IN Genomes (TILLING) [64]. Minoia et al. [68] obtained a new mutant collection at domestication by EMS. All of the genome scans identified 66 nucleotide substitutions and reported two different mutation intensities. In Barley, two gene-induced mutations were generated using EMS and the results were confirmed by sequence analysis [69]. In another study conducted at barley, 63 androgenic doubled-haploid mutants were obtained by sodium azide application during anther formation in vitro [70]. In the study by Kim et al. [71], homologous mutant lines were developed resistant to 5-methyltryptophan (5MT) in rice. In a similar study in rice, mutants which were resistant to 5-methyltryptophan (5MT) were also developed, and the amount of both protein and nine free essential amino acids increased significantly from the original variety [72]. In another study on rice, a new mutant genotype with high tocopherol content was obtained in in vitro mutagenesis with gamma irradiation. Mutant individuals were found to have higher seed viability than the control group and seedling growth was faster in the early growth phase [73]. Induced mutation treatment resulted in acidity and drought tolerance in lentil and rice [74–76]. Again, in a study of rice, salt tolerant varieties were obtained by mutation induction [77]. As seen in all of these studies, many mutagenesis applications have been made in order to improve plant characteristics and to create genetic diversity, and successful results have been achieved. Some methods are used to detect the regions of mutation and density. Methods such as conformation-sensitive capillary electrophoresis (CSCE), single-strand conformation polymorphism (SSCP), and denaturing high performance liquid chromatography (dHPLC) are used to determine variations in plant genes. In addition to these methods, TILLING and High-resolution melting (HRM) are used to determine the induced polymorphism [25, 65, 67].

5. Conclusion

Mutations naturally occurring in the evolutionary process led to the formation of new genotypes. Mutations that occur in nature spontaneously have been modeled for humankind in order to increase the genetic diversity, which is narrowed as a result of natural selection and classical breeding studies. Thus, induced mutation studies have begun. Mutation induction studies, which provide new alleles to the genome by different methods, contribute to the increase of genetic diversity. The increase in genetic variation will increase the chance of survival of species in changing biotic and abiotic conditions. Genetic diversity is not only important for the continuity of species, but also improves the quality criteria of plants, which are raw materials of many industrial products such as food, pharmaceutical, textiles, etc., in these sectors.

Conflict of interest

The authors declare that they have no conflict of interest.

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