Chapter

New Frontier of Plant Breeding Using Gamma Irradiation and Biotechnology

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Abstract

Mutation is an underlying cause of evolution as a mutant, either natural or artificial, with a novel trait may be preferentially selected for nature because of its superior survival adaptive features. Because of the desirability of the novelty, mutation is the heritable change to an individual's genetic makeup, which is passed on from parent to offspring and thereby, drives evolution. In nature, mutations are spontaneously caused by errors in the DNA replication. Gamma radiation induced mutation in plant breeding is the one effective method that can cause DNA changes via direct and indirect actions. Many crop varieties have been created using gamma irradiation mutagenesis technology for trait improvement that enhance the characteristic or increase the abiotic and biotic stress tolerance. Plant breeding and genetics procedure usually start from mutation induction by gamma irradiation and work with the other modern enabling technologies, such as tissue culture or molecular genetics. Tissue culture and bioreactor techniques are used for synthesizing new plant varieties, while the molecular genetic technique is used for genetic analysis of the new varieties. The irradiation coupled with new modern tissue culture and molecular genetic technology is widely used to induce plant mutation breeding for creating new commercial plant varieties.

Keywords: plant mutation breeding, irradiation, biotechnology, tissue culture, molecular genetic

1. Introduction

Nowadays, agriculture has changed dramatically compared to the past. Technologies have played an important role that can be applied to use in almost every aspect of the agricultural value chain from production to consumption. The world population is currently around 7.9 billion and is expected to reach 10 billion in 2050 [1]. To feed the expected world population, the production of food will need to increase to meet the global food demand. Improved agricultural

production through plant breeding, is one of the key solutions to sustainable development for supporting the growing global population. Plant breeding refers to the development of plant cultivars suitable for agricultural cultivation. In nature, natural selection is responsible for the survival of plants, based on their characteristics. Plants with low survival quality would be eliminated naturally. High-quality plants can be crossed and matched up using farmers' knowledge and expertise. Gregor Mendel, the modern genetics pioneer, did research based on the principles of genetic theories. Later, radiation and chemical mutagens were developed to help induce genetic mutation more rapidly than natural evolution.

2. Mechanism and types of mutation

2.1 Mutagenesis mechanism

Mutations involve changes in the genetic material (DNA) of cells and can be passed on to offspring through the cell division process. Mutations may be associated with the loss of a gene or structural changes within a gene, which is called gene mutation or point mutation. Chromosome-related mutations can involve changes in the number of chromosomes, exchange of chromosomes, and the disappearance/addition of a segment of a chromosome.

1. Gene Mutation

Gene mutation or point mutation is a chemical change in a gene involving only a few nucleotides bases in a gene that affects some genetic changes in that gene.

2. Chromosome Mutation

Chromosome mutation is the change in the number or the structure of the chromosomes.

a. Changes in the Structure of Chromosomes

A missing or duplication part of the chromosome can cause a number of genes to be changed as well. The effects of these changes vary greatly depending on the type of gene and the number of genes involved.

b. Changes in Chromosome Number

Usually, plants have diploid chromosome numbers, an increase or decrease can occur only on certain chromosomes called aneuploidy, for example, $2n + 1$ or $2n - 1$. In case of increase or decrease of chromosomes sets, it is called polyploidy.

2.2 Type of mutagens

Mutagen can be divided into three types, which are as follows:

1. Physical mutagen: Radiation such as X-rays, gamma rays, ion beams, electron beams, and neutron particles.

2. Chemical mutagen: Chemicals that cause mutations in genes or chromosomes. These chemical substances are ethyl methane sulphonate (EMS), diethyl sulfate (dES), ethyleneimine (EI), N-ethyl-N-Nitrosourea (ENU).

3. Biological mutagen: Genetic disruption might be the result of the virus and bacteria.

Comparatively, gamma and X-ray irradiation can be used to induce changes in seeds or other reproductive organs of the plant, such as branches, buds, stems, bulbs, and corm because they can penetrate into the internal tissues better than chemicals and also the method does not lead to chemical waste [2].

3. The use of radiation-induced mutations

It has been over 100 years since Darwin developed the theory of evolution by natural selection. Many scientists have learned and discovered the evidence of this theory that relates to a genetic mutation. Furthermore, they have found ways to induce and utilize mutations. The development of genomic study helps enhance the plant mutagenesis in crop improvement more efficiently. The mutant traits, such as dwarfing, sterility, disease and pest resistance, change of metabolites content, and many others, were shown in many species from crops to ornamentals by mutation induction. The need for plant breeding has been demonstrated usually by combining genetic modifiers to change the original mutant phenotype to become a significant breeding target. The mutagenesis technique is one of the apparatus that has resulted in many impressive mutant varieties. The number of such varieties is high across economic plant species, including vegetatively propagated species [3, 4].

Mutation techniques have been used widely in efforts to create new plant varieties that are more resistant to biotic and abiotic stress. The effects induced by physical and chemical mutagens can be similar to the natural mutation in major crops. The use of *in vitro* mutagenesis combined with *in vitro* selection with the parental lines has significantly improved the plant mutation breeding efficiency, especially in bananas and potatoes [5]. The success of mutation breeding depends on three main factors—mutagenesis efficiency, the starting plant material, and mutant screening. Identification of the genes associated with valuable traits would help in understanding the genetic background and choosing suitable starting material by molecular genetic technologies. Finally, the efficiency of mutant screening will impact the cost of mutation breeding, especially in plant species requiring long periods before the mutant traits can be evaluated [6].

Genetic variation is a prerequisite for plant breeding required to obtain useful traits for crop improvement. Often, the desired variation is either decreasing or disappearing over time. Moreover, spontaneous mutations occur at a very low rate that cannot be used in plant breeding for creating and developing new plant varieties. Therefore, the increase in mutation rate needs to be enhanced by the induction of genetic variability using mutagen treatments, such as gamma-ray, X-ray, fast neutron, ethyl methane sulfonate (EMS), and sodium azide, among others.

Plant breeders recognize the importance of ionizing radiation in plant breeding research programs. The technique has been tried out since 1930 and became accepted around 1950 because it solved some problems that standard conventional plant breeding methods could not. The two main advantages of irradiation are—1) some crops, especially cereal crops, have undergone extensive breeding that in some areas and could not be advanced from existing germplasm or strains, some characteristics, such as disease resistance, were rarely found in natural plants. 2) the increase in world population destroyed native plants because the farmer's plant only improved the cultivation of plant species that have a disadvantage in terms of having a narrow genetic background. The use of radiation induces mutations in plants is one way to solve the problem of variability in plant species by inducing the desired characteristics that are not found in genetic sources. Ionizing radiation, such as X-rays, gamma rays, and neutron rays, can cause damage to genetic material and lead to phenotypic changes. Any propagated parts of plants can be irradiated, including seeds, buds, shoots, and stolon. However, seeds are most commonly used and most convenient. Radiation-induced mutations occur randomly, but their rate of occurrence could be made higher than spontaneous mutations. Evolution and practical plant mutation breeding both depend on genetic variation. Desired characteristics are selected and the obtained mutants are investigated and screened criteria essentially include the direct or integrated study of morphological variability, alteration of physiological and biochemical parameters, gene expression analysis, etc., under different degrees of stress conditions. Before conducting any plant mutation breeding experiment, one has to define the desired characteristics. The screening for such characteristics can be done *in vitro* and/or *in vivo* depending on the species, trait/character (abiotic stresses) understudy, biotic/abiotic factors influencing the expression of the trait(s), etc.

4. Technology in mutation breeding and tissue culture

4.1 Induced mutation in the plant by irradiation

Plant breeding is the development of plant species to have better characteristics than the original variety and to meet human needs. Plant breeding can be used for many different purposes, such as to increase productivity, nutritional value, to withstand inappropriate cultivation conditions disease and insect resistance, for enhanced appearance, etc. Plant breeding methods are as follows.

4.2 Conventional plant breeding

Conventional plant breeding is the development or improvement of plant varieties by using conservative tools for manipulating plant genomes within the natural genetic boundaries of the species. The common methods for breeding self-pollinated species include mass selection, pure line selection, pedigree, bulk population, single seed descent, backcrossing, multiline and composite [7].

4.3 Induce mutation plant breeding

Natural mutations may occur, whereas at low rates and over a lengthy period. In this way, the mutation's induction takes less time than a spontaneous mutation. To induce mutations, mutagens must be used. The most commonly used mutagens are

chemicals, but it is not safe for workers and the environment. Irradiation is another popular mutagen used to induce mutations.

4.4 Induced mutation in the plant by irradiation and tissue culture

Advantages of plant propagation by tissue culture are as follows:

1. It can increase the volume of plants in large quantities in less time than normal propagation. This saves space and labor used for propagation.

2. Disease-free plants can be obtained. Diseases are caused by fungi or bacteria infecting seeds or other propagation. In tissue culture, plant parts are sterilized before being cultured on a tissue culture medium. If there is mold and bacteria on it, it will show and can be removed.

The process of induced mutation in plants by irradiation and tissue culture

1. Study the appropriate tissue culture medium.

Plant tissue culture should be studied in a formula that is appropriate for the species and parts of the plants tested to enable the plants to grow well. and expand the number appropriately with strong plants.

2. Irradiation

Two methods of irradiation combined with tissue culture can be performed [8]

a. Irradiated plant parts such as seeds, lateral buds, shoots, and branches. Then the plant parts are sterilized and tissue cultured. The disadvantage of this method is that the plant becomes contaminated with microorganisms after tissue culture, resulting in less plant tissue than in reality.

b. Irradiated plant parts, such as seeds, lateral buds, shoots, and branches. Then the plant parts are sterilized and tissue cultured. The disadvantage of this method is that the plant becomes contaminated with microorganisms after tissue culture has fewer plants than the number of plants that should have been the chances of selecting a mutant will also be less.

Irradiation can be done in two ways: 1. Acute irradiation is the irradiation of a high dose in a short time or irradiation at a high dose rate, 2. Chronic irradiation is the irradiation of a low dose and uses a long time or irradiation at a low dose rate (**Figure 1**).

Plant tissue before irradiation is called M_0V_0, with M standing for meiotic and V for vegetative, when irradiated, it is called M_1V_1 generation. New plants or new shoots from M_1V_1 generation are called M_1V_2 generation. It can be subcultured to the next-generations, called M_1V_3, M_1V_4, M_1V_5, and so on (**Figure 2**).

3. Selection of mutations by tissue culture technique

After irradiation of plant tissue, the tissue is subcultured to M_1V_2 generation. In M_1V_2 generation, there can be genetic differences in new plants or new shoots that can

Figure 1.
(left) Gammator and (right) Gamma room at Thailand Institute of Nuclear Technology (Public Organization).

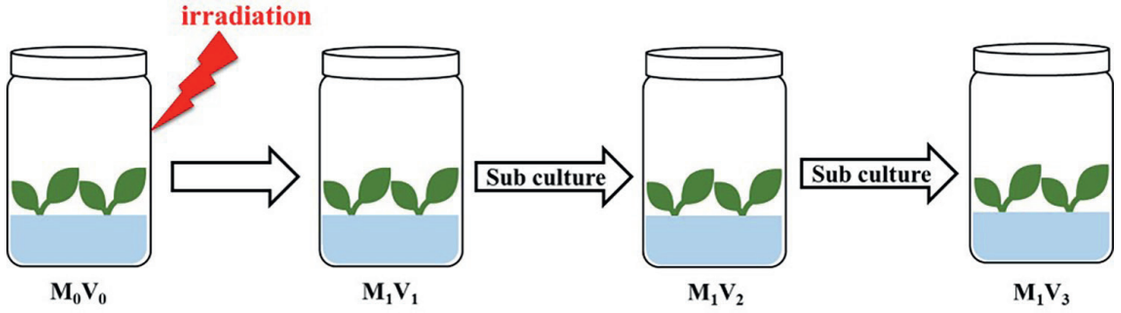

Figure 2.
M_1V_0 - M_1V_3 generation.

be divided into three groups: 1. The original species, 2. The group of tissues that are all mutated is called a solid mutant, and 3. The group in which the mutated and normal tissues are combined is called a chimera [8]. The occurrence of chimera in different layers of plant cells affects different plant phenotypes, as illustrated in **Figure 3**.

The size and frequency of sectoring are a function of transposon (or other mutagens) activity and the rate of cell division [9]. Tissue culture can help in the separation of mutated and normal sections by subculture. The mutated section is cultured, and the subculture continues until the solid mutant is obtained (**Figure 4**).

Examples of research on plant breeding by radiation in combination with tissue culture.

Study on the effect of gamma irradiation on tissue culture of aquatic plant "*Anubias nana*." It was found that the selected mutants in the M_1V_4 generation had variegated leaves, dwarfism, light green leaves, abnormal leaves, and albinism (**Figure 5**).

Mutation's breeding on tissue culture of Curcuma hybrid "Laddawan." The experiment revealed that morphological variations in the M_1V_2 generation were observed at 50 Gy gamma irradiation treatment samples with variegated leaves and light green leaves were observed (**Figure 6**).

The use of tissue culture techniques to assist in the selection of mutations in tissue culture of bromeliad "*Tillandsia cyanea*". The selected mutants in M_1V_3 generation had light green leaves, variegated leave, dwarf, and giant characteristics. The most common characteristic was light green leaves (**Figure 7**).

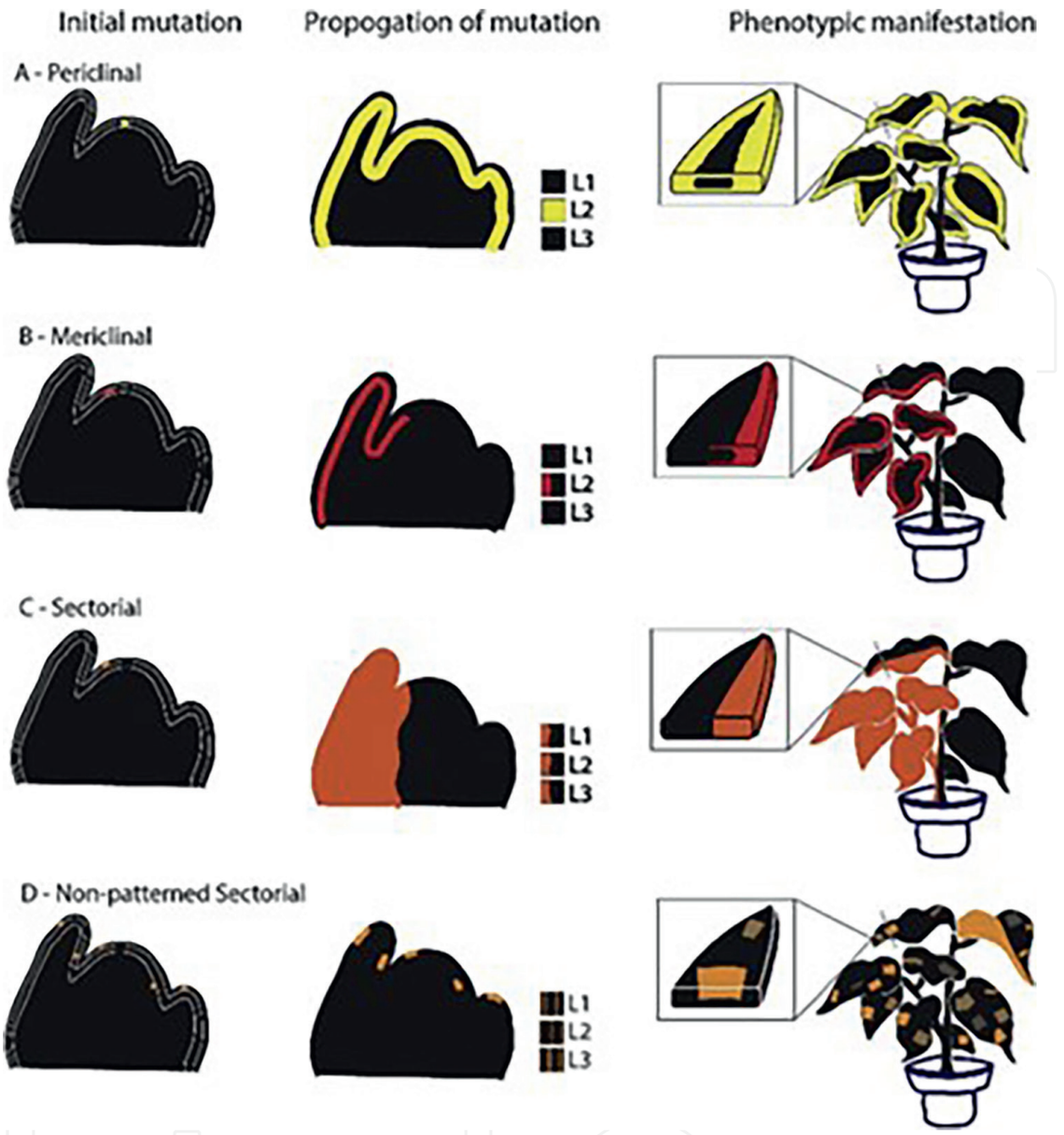

Figure 3.
Shows mutation characteristics and phenotypic expression. (A-B) has a mutation at the L2 layer (shown in yellow and red, respectively) causing phenotypic mutation along the leaf margins. (C-D) has a mutation at L1, L2, and L3 layers, which are often characterized as being unstable. (C) Sectorial are traverse all layers of the shoot meristem. (D) Non-patterned sectorial are variegated appearance.

Besides the traditional tissue culture method, another tissue culture method may increase the number of plant tissues in a shorter time. This is called the TIB (Temporary Immersion Bioreactor) System. This system has a container that separates the liquid tissue culture medium and plant parts into two parts, each with a tube connected to allow the liquid tissue culture medium to be pushed back and forth with air pressure from an air pump. The condition inside the bottle was aseptic by filtering the air entering the bioreactor with a filter with a pore size of 0.2 μm plant This prevents the plant from drowning in liquid food all the time [13].

The working principle is divided into four phases as follows [14]:

1. Stationary phase: Systemic tissue is normal in culture parts and media parts with liquid food.

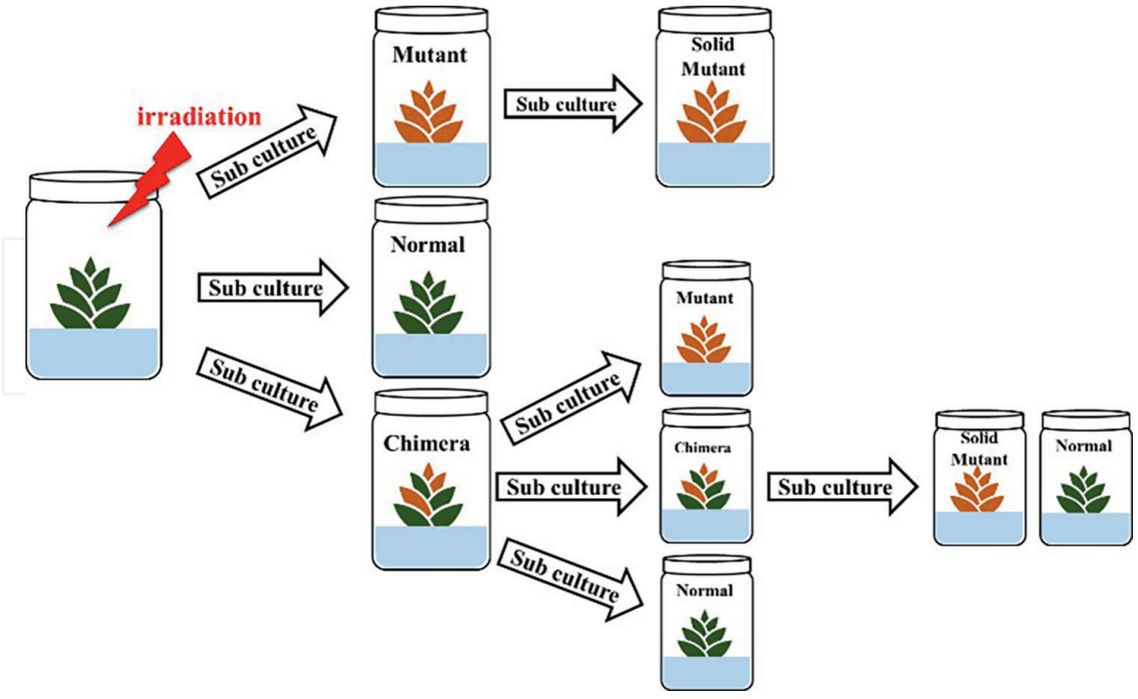

Figure 4.
Selection of mutations by tissue culture technique.

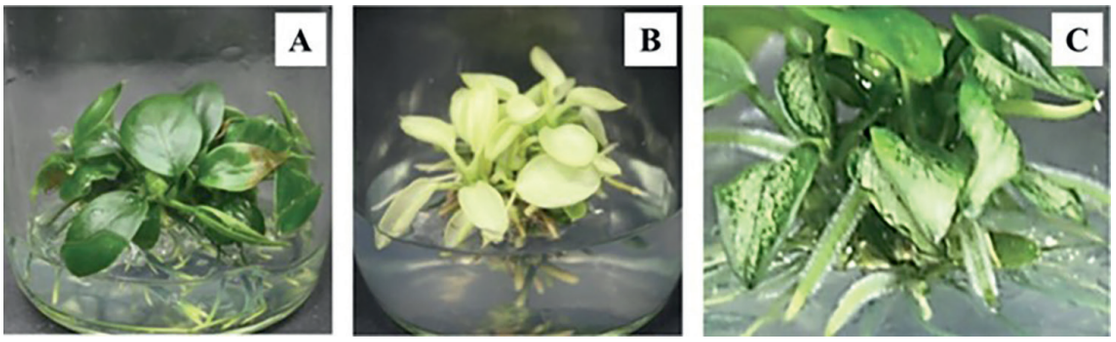

Figure 5.
Characteristics in the M_1V_4 generation of Anubias nana, (A) normal plant, (B) albinism, and (C) variegated leaves [10].

2. Immersion phase: It is the stage of air pressure to deliver liquid food to flood the plant tissues in the culture part at the specified time

3. Drain phase: Allowing liquid food to flow back into the vessels below by gravity.

4. Ventilation phase: The phase of air pressure into the vessels above (**Figure 8**).

The temporary immersion bioreactor system is a fast automated system. Therefore, the use of labor in the work is reduced. In addition, the container capacity of the bioreactor system temporarily sinks and can increase by 4–5 times. Thus, increasing the volume per area can also reduce the area of the tissue culture room down. Therefore, the temporary immersion bioreactor system is a new and suitable plant propagation system. It can replace the traditional plant tissue culture system (solid medium) and can be used in industrial plant production [15].

Figure 6.
Characteristics in the M_1V_2 generation of Curcuma hybrid 'Laddawan', (A) normal plant, (B) variegated leaves, and (C) light green leaves [11].

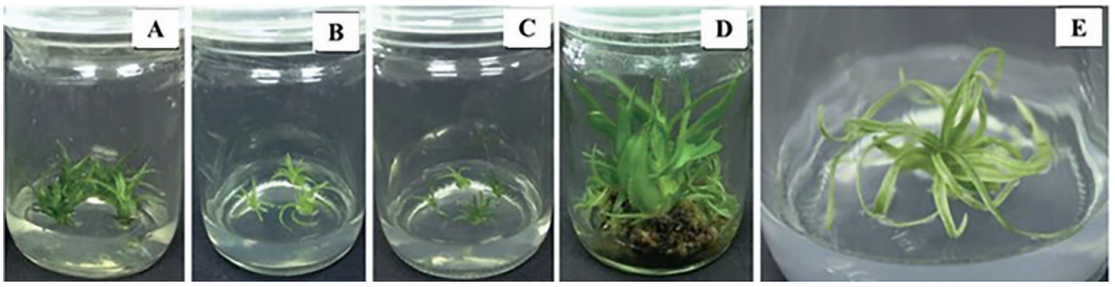

Figure 7.
Characteristics in M_1V_3 generation on tissue culture of Tillandsia cyanea after acute gamma irradiation, (A) normal plant, (B) light green leaves, (C) dwarf, (D) giant, and (E) variegated leaves [12].

Figure 8.
Bioreactor, (top) vertical sample vessels, and (below) horizontal sample vessels.

5. Application of molecular biology techniques for plant breeding

According to the article mentioned above, chemical and physical mutagens can cause genetic material alteration and have been widely used in plant mutation breeding. Variety identification or classification is an important step for plant breeding including the investigation of genetic variation and relatedness which are critical aspects of the maintenance of biodiversity. The identification method often uses genetic markers as a specific identifier that have been developed and used as a modern technique for characterization genotypes of organisms. A genetic marker is divided into three types [16–21].

1. Morphological marker

This marker is a physiological indicator that can be observed through general appearance and can be observed in morphological traits, such as plant height, canopy width, flower color, size or shape of the flower, flowering time, harvesting time, etc. It may be called naked-eyed polymorphism. These traits are mainly expressed by the controlling gene, so they can be used as a genetic marker. However, this marker has limited use, due to the morphology of plants often varying according to the changing environment, thus, the observed traits may cause errors in species identification. In addition, some plants are closely related species, the external characteristic comparison cannot be easily distinguished. Therefore, other indicators are required to improve the species classification accuracy.

2. Protein marker

The development of protein markers is to help identify the differences of plant species using different protein molecules which are components of plants to be examined. Protein markers are widely used to verify genetic purity for seed production. However, protein markers also have important limitations. The number of genes or proteins used for testing is small and genes that use for investigation must be expressed. Therefore, the investigation is necessary to select the optimal plant tissue and growth stage from gene expression. Moreover, the expression effect depends on the environment as well. This results in increasing the chance of the variation detecting in protein levels being undervalued. In addition, the use of protein markers in plant species detection is also limited because proteins are products of gene expression. It was found that the environment influences the expression of genes. If the environment is not suitable for plant growth, some genes cannot be expressed and proteins cannot be synthesized. Therefore, the results may vary and the classification between species that are closely related to each other can be impossible.

3. DNA marker

DNA marker is a DNA sequence that is used as a unique marker of an organism and can be inherited by the next generation. Each plant species has its own unique arrangement of nucleotides or polymorphisms of the DNA molecule that makes difference. So, it can be used as a marker to construct DNA profiles and assign the breeding lines to the various heterotic groups as well as variety identification. Using DNA as a marker to identify varieties of organism genetic material pattern which has a unique pattern to each individual can be done by a molecular and genetic technique which are commonly known as DNA profiling or

DNA fingerprint. DNA markers can be classified into three types according to the principles and techniques used for development.

- Hybridization based marker

This marker is developed based on the hybridization principle, which is the process of combining DNA probe and DNA target with complementary base pairs, such as restriction fragment length polymorphism (RFLP).

- Polymerase chain reaction-based marker

DNA markers are developed based on the principle of the rapid amplification of DNA as known as polymerase chain reaction (PCR), such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), inter simple sequence repeat (ISSR).

- Sequence-based marker

A developed marker based on the identification of nucleotides in DNA, such as single nucleotide polymorphism (SNP) (**Figure 9**).

Sometimes protein markers and DNA markers can refer to molecular markers. Molecular markers are a very useful tool to assess genetic diversity and relationships. The application of molecular markers in plant mutation breeding can help to accelerate the breeding program, increase accuracy and save cost and labor. They can apply to the study of genetic diversity, phylogeny, polymorphism analysis, and cultivar identification [24–28]. These markers have their own advantages and disadvantages, the appropriate marker selection depends on the purpose of use, the specific of each marker, facility, financial, time and knowledge. The comparison of some widely used DNA markers is shown in **Table 1** [19, 29, 30].

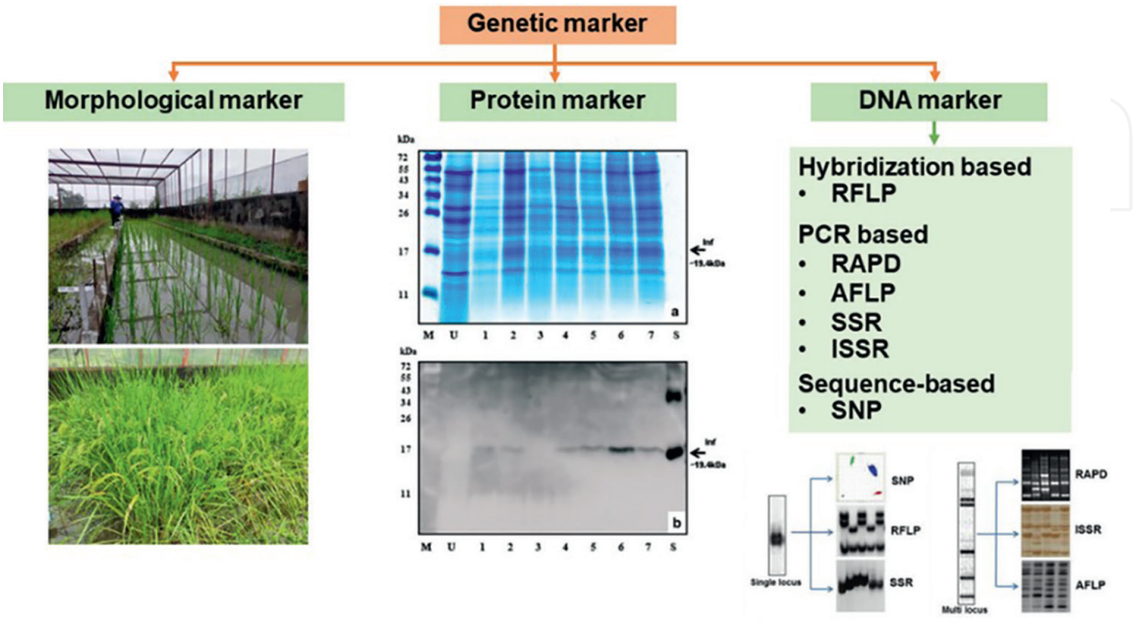

Figure 9.
The overview of genetic markers [22, 23].

Technique	RFLP	RAPD	AFLP	SSR	ISSR	SNP
Amount of required DNA	10 μg	20 ng	50-100 ng	50 ng	50 ng	50 ng
Quality of required DNA	High	High	High	Low	Low	Very high
Reproducibility	High	High	Medium	High	Medium-High	High
Genome abundance	High	Very high	Very high	Medium	Medium	High
Polymorphism level	Medium	Very high	High	High	High	High
Cost	High	Low	High	High	High	Variable

Table 1.
Comparison of some widely used DNA markers.

The development of DNA technology has grown rapidly over the past 10 years. There is a lot of new knowledge that can be applied widely in agriculture especially in plant breeding with an important purpose to create new varieties with good characteristics, such as high yield, disease resistance, pest resistance, and unfavorable conditions resistance. DNA markers are constantly being developed. It is widely used to identify the difference or diversity of organisms whether in terms of quantity or quality trait and is applied as a tool by plant breeders in many fields. Examples of the application of DNA markers in plant breeding are identification and purification of plant varieties, genetic assessment, genetic linkage mapping construction, and gene mappings, such as quantitative trait locus (QTL) and marker-assisted selection (MAS) [31–35]. DNA markers can distinguish more accurately and precisely than using plant morphology which often varies according to the environment. While protein marker is also limited, as it is based on gene expression. The advantages of DNA markers are as follows:

1. High accuracy – DNA marker is a tool that directly selects the desired plant genotype. Therefore, it is more accurate and precise than the selection from phenotypes that are related to the environment.

2. Large number of markers available for use

3. Constant results – The use of DNA marker to help in cultivar selection can be performed at every stage of plant growth and can use seed or tissue from any parts to be examined due to DNA remaining unchanged. Therefore, plant breeders can select the mutants at an early stage, which shortens the selection process. That plant can continue to grow. This is useful in cases when MAS is used because

4. Nondestructive – This is because only a small part of the plant is collected for DNA extraction and examined using the DNA marker. The interested mutant that is investigated can continue growing. There is no need to replant.

5. Inheritance classification – Some DNA markers, RFLP and SSR, are codominant markers that can be distinguished by both heterozygous and homozygous genotypes. While some DNA marker, such as RAPD, is not possible to distinguish between heterozygous genotype and homozygous genotype.

6. Able to select many desired characteristics simultaneously – The use of DNA markers can help select multiple desired traits at the same time and during the early growth stage. It saves time, labor, cost, and planting area.

6. Application of gamma irradiation on crop varieties

Rapid population growth, environmental pollution, and climate change have greatly affected human survival on Earth. The unsustainability of food, medicine, herb, and fuel supply chains is becoming a major problem for people around the world. Agricultural crops are very important to provide food, which is one of the most basic needs of human beings. How to feed the world's consumption poses great challenges to farmers and policymakers. To date, the method of artificial mutagenesis to obtain new biological cultivars, therefore, becomes a major challenge for breeders, and developing strategies to increase the genetic variability has demanded the attention of several research groups. The first paper on the technology of irradiation for mutation breeding employed X-rays in inducing mutations in maize and barley by Stadler in 1928 [36]. The first commercial mutant crop generated by irradiation technology is *Nicotiana tabacum* or "chlorine type" [37]. The leaves of these plants are used in the production of cigars, chewing tobacco, and nicotine replacement products. Plant breeders have been encouraged to use mutation breeding as one of the "peaceful uses of atomic energy." The main strategy in mutation-based breeding has been to upgrade the plant by altering traits to enhance their productivity and quality. In 2022, there are more than 3,391 cultivars developed from mutation breeding and registered in FAO/IAEA mutant database (**Table 2**). The mutant varieties database was split into three categories, crop plants, ornamental plants, and others were 47%,

Category	Records
Crop plants	1,592
Ornamental plants	711
Others	1,088
Total	3,391

Table 2.
The categories of FAO/IAEA mutant varieties database.

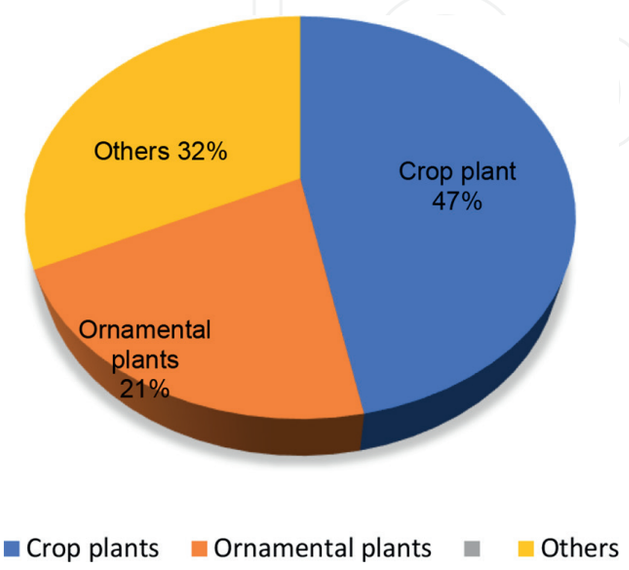

Figure 10.
Pie chart showing the percentage of categories plant data on FAO/IAEA mutant variety database.

21%, and 32%, respectively (**Figure 10**). Among these new varieties, about 1,703 cultivars were induced by gamma-ray irradiation [38].

Gamma irradiation mutagenesis technology has been widely used in crop mutation for a long time, partly due to restrictions on other techniques, such as hybridization, cross-breeding, and transgenic plants. Gamma radiation is able to penetrate the target material thoroughly and cause DNA damage in the chromosome. The DNA damage is difficult to repair effectively and correctly, leading to the generation of mutant plant varieties. To date, the IAEA reported that there are nearly 300 gamma irradiators from around the world [39].

Many crops, such as food, herbal medicine, flower, and ornamental, have been created using gamma irradiation mutagenesis technology for trait improvement. This can support food security, through enhanced characteristics, or increased resistance to environmental stress. Many plant varieties have been developed using gamma irradiation and have been released and widely cultivated worldwide.

Food crops are most important for human survival. Many countries around the world are looking for techniques to improve crop diversity. The mutagenic effects of gamma-rays in plants have been a particularly important issue concerned by the breeders. There are many mutated crops varieties were reported in the FAO/IAEA database (**Table 3**). Wheat, rice, and soybean are the most important crops for the breeders, about 22%, 19%, and 15%, respectively, resented in mutated crop varieties in the database (**Figure 11**). The strategies for genetic improvement of the crops are the increase in production, improvement of nutrition, and higher resistance to unfavorable conditions, such as drought and salinity. However, the high-yield crops are considered by the breeders. Both total doses, dose rate and multiple biological endpoints on crops after exposure to gamma-ray were investigated. The rice seeds of the non-waxy variety 'Toyonishiki' were exposed to 20 kR of gamma-ray. Two out of 20,000 panicles produced on these plants had waxy grain, and one of these brought forth a new commercial variety, "Miyuki-mochi." The yield of the new variety was

Common name of crop plants	Records
Wheat	265
Rice	230
Soybean	182
Barley	106
Maize	89
Groundnut	79
Mungbean	39
Chickpea	27
Pea	30
Lentil	19
Sugarcane	13
Sorghum	18
Others	117

Table 3.
List of mutated crop plant varieties from FAO/IAEA mutant varieties database.

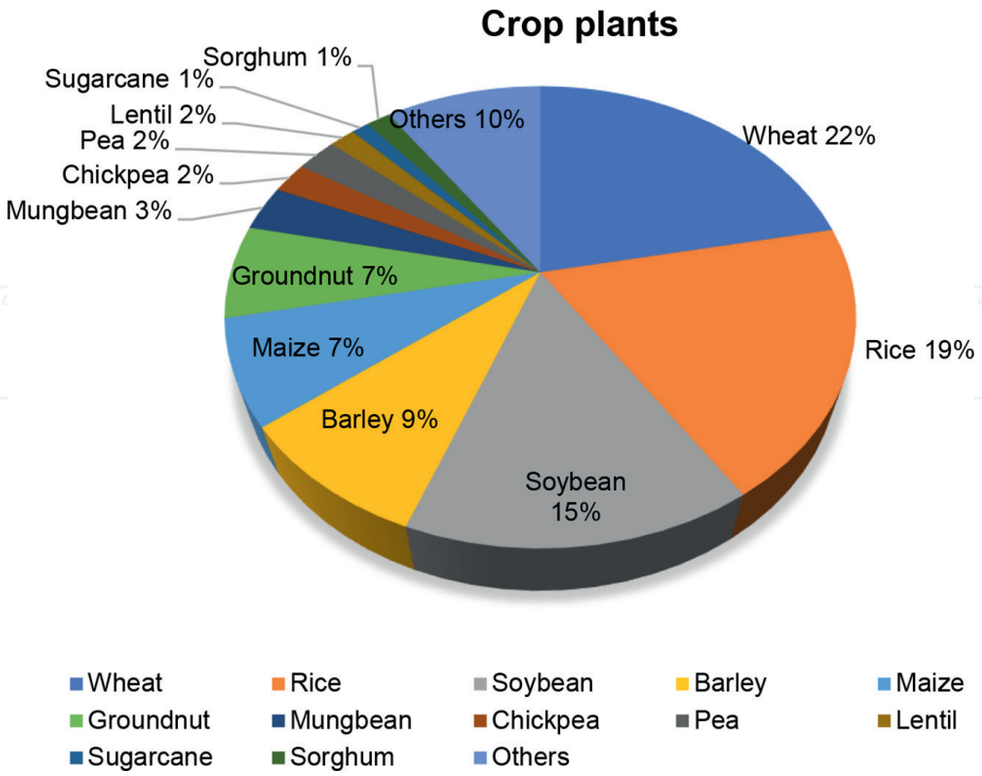

Figure 11.
Pie chart showing the percentage of crop plants on FAO/IAEA mutant variety database.

reduced by 8% as compared with the original variety, but the heading date, culm length, panicle length, and the number of panicles of the new variety were almost the same as those of the original variety. If compared with the old leading waxy variety, "Shinano-mochi," "Miyuki-mochi" is superior in yield by 15% and has high resistance to lodging and rice blast [40]. The seeds of five Japanese rice cultivars were irradiated with 250 Gy of gamma-ray (over 20 hr) and five high-yielding mutants were obtained [41]. High salinity in the soil is one of the major abiotic stresses leading to the reduction of rice yield. The seeds of "Dongan" rice were exposed to gamma-ray and selected for salt tolerance. They introduced the systemic procedures for the selection of salt tolerance rice plant mutants, and two promising mutant lines, ST-87 and ST-301, were finally selected [42].

Medicinal plants are important for disease prevention and in human healthcare for a long time. To date, numerous studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines. The global market value of medicinal plant products exceeds $100 billion per annum [43]. Medicinally important plants are enhancement of secondary metabolite production, both *in vivo* and *in vitro* by gamma irradiation is a current area of interest**.** In the common herb babchi (*Psoralea corylifolia*), phenolic compounds in whole plants/plant parts irradiated with γ-ray at 20 kGy increase as high as 32-fold. The capsaicinoids, a phenolic compound in paprika (*Capsicum annum L.*) increased by about 10% with 10 kGy. The *in vitro* studies show all three types of secondary metabolites are reported to increase with γ-irradiation. Stevioside, total phenolic, and flavonoids content were slightly increased in 15 Gy-treated callus cultures of stevia (*Stevia rebaudiana* Bert.). In terpenoids, total saponin and ginsenosides content was increased 1.4- and 1.8-fold, respectively, with 100 Gy for wild ginseng (*Panax ginseng* Meyer) hairy root cultures.

In alkaloids, camptothecin yield increased as high as 20-fold with 20 Gy in callus cultures of ghanera (*Nothapodytes foetida*). Shikonins increased up to 4-fold with 16 Gy in suspension cultures of purple gromwell (*Lithospermum erythrorhizon S.*) [44].

Flowering plants and ornamental plants are two types of plants held in a special place in the history of humanity since ancient times. The sight of beautiful fresh flowers has a positive effect on the viewer. The use of such plants for decorative and aesthetic purposes has grown in floriculture. The report on the global market provides

Common name of ornamental plants	Records
Chrysanthemum	285
Rose	67
Dahlia	36
Carnation	28
Alstroemeria	35
Begonia	18
Azalea	15
White lupin	14
Hibiscus	14
Tulip	9
Achimenes	8
Canna lilies	8
Scotch broom	9
Lily	6
Others	82

Table 4.
List of ornamental plant varieties from FAO/IAEA mutant varieties database.

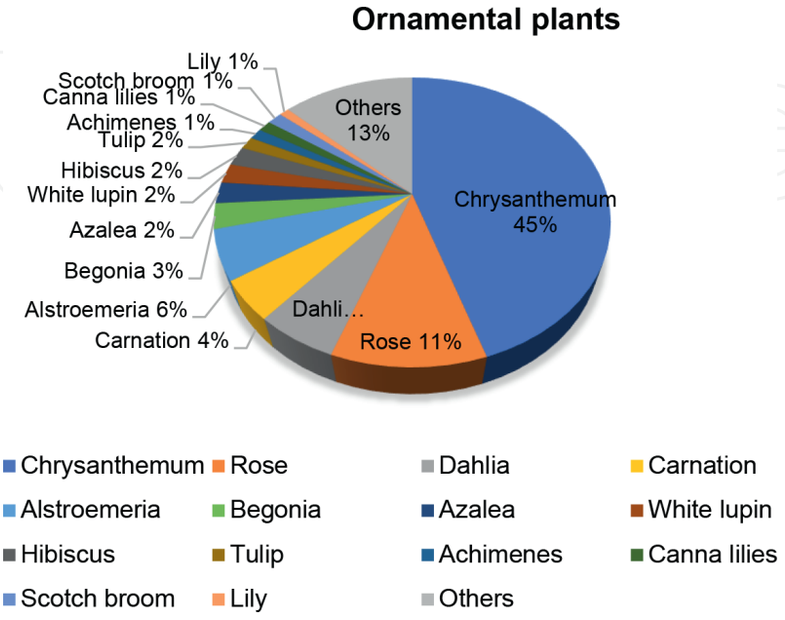

Figure 12.
Pie chart showing the percentage of ornamental plants on FAO/IAEA mutant variety database.

a holistic analysis, market size and forecast, trends, growth drivers, and challenges of flower, and the ornamental plant has been closely monitored and it is poised to grow by 27.18 billion dollars during 2021–2025 [45]. The mutation of flowering and the ornamental plant has become more successful owing to additional changes in phenotypic characteristics, heterozygous nature, and high mutation frequency producing a large number of new plant varieties. Gamma irradiation mutagenesis was used to improve the phenotype of flower and ornamental plants for a long time. In 1954, the first mutant propagated variety was released as a tulip variety with better flower shade and arrangement [46]. To date, there are various ornamental plants were recorded in FAO/IAEA mutant varieties database (**Table 4**). The three most of release mutant plants were chrysanthemum, rose, and dahlia (**Figure 12**). During 1970–1997, FAO/IAEA mutant varieties database shows the list of officially released

Scientific name	Common name	Mutant variety	Country of release	Year of release	Main improved characters
Dhalia sp.	Dhalia	Adagio	France	1970	Flower color
		Bichitra	India	1978	Plant architecture
		Huanghuan	China	1978	Shortness
		Jyoti	India	1978	Plant architecture
		Meiguizi	China	1989	Flower color
		Twilight	India	1978	Plant architecture
Dianthus caryophyllus	Carnation	Bonitas	GDR	1985	Semi dwarfness
		Chaichompon	Thailand	1983	Flower color
		Galatee lonvego	France	1982	Fusarium
		Loncerda	France	1983	Fusarium
		Maiella lonchabi	France	1982	Fusarium
		Scrlet Bell	Japan	1983	Flower color
		Sim Feu Follet	France	1972	Flower color
Gerbera jamesonii	Gerbera	Raisa	Poland	1993	Flower color
Gladiolus sp.	Gladiolus	Shobha	India	1988	Flower color
		Tambani	India	1991	Flower color
Hibiscus sp.	Hibiscus	Anjali	India	1987	Flower color
		Purnima	India	1979	Variegated leaves
Nelumbo nucifera	Lotus	Dandinyuge	China	1997	Flower color
		Dianezhuang	China	1983	Earliness
Rosa sp.	Rose	Beijingzhichun	China	1990	Flower color
		Beiyumudan	China	1986	Flower color
		Bridal Sonya	Japan	1985	Flower color
		Caiyemingxin	China	1986	Leaf morphology
		Paula	USA	1960	Flower color
		September wedding	Canada	1983	Flower color

Table 5.
List of officially released mutant flowering and ornamental plants from FAO/IAEA mutant varieties database [47].

Common name	Scientific name	Applied dosages	Treated materials	Results
Orchid	*Dendrobium sonia*	10 to 200 Gy	Protocorm-like bodies	Untreated orchids' survival rate and weight were higher compared to treated plants
Tuberose	*Polianthes tuberosa*	5, 10, 15, 20, 25, and 30 Gy	Bulbs	Variegated leaves and mutant cultivars were found with 20 Gy
Tuberose	*Polianthes tuberosa*	2 and 4 KR	Bulbs	Early sprouting, early spike emergence, and 50% flowering were noticed at 2 kR than 4 kR
Gladiolus	*Gladiolus grandifforus*	15, 30, 45, and 60 Gy	Corms	Low doses of gamma irradiation encouraged the vase life of a flower and changed floret colors
Gladiolus	*Gladiolus hybrida*	0.5 to 5.0 kR	Corms	Earliest sprouting was observed in 3.0 kR
Gladiolus	*Gladiolus spp.*	25, 40, 55 and 70 Gy	Corms	Spike length, number, and size of florets were decreased at 70 Gy
Gladiolus	*Gladiolus hybrida*	1 to 7 kR	Corms	2 and 3 kR proved better stimulation overall treatment
Gerbera	*Gerbera jamesoni*	1.5, 2, 2.5, 5, 10, 15, 20, 30 Gy	In vitro shoots	In radiated plants with 5 Gy doses showed moderate resistance to powdery mildew
Hibiscus	*Hibiscus Rosa-sinensis*	10, 15, 20, and 25 Gy	Nodal segments	5 Gy was effective for controlling Phytoplasma and the survival rate of plants
Marigold	*Glebionis segetum*	20, 40, 60, 80, and 100 Gy	Seeds	20 Gy changed disk color and 40 Gy changed floret and disk color
Sunflower	*Helianthus annuus*	100 to 900 Gy	Seeds	100 and 200 Gy have a positive effect on plant height and root length, LD 50 have found at 500 Gy
Blushing philodendron	*Philodendron erubescens*	70, 100, 150Gy	Rooted cuttings	The color composition of leaves ranged from 0–10% dark bluish-green, 60–90% strong yellow-green and 10–30% brilliant greenish-yellow
Gerbera Daisy	*Gerbera jamesonii*	10, 20, 30, 40, 50, 60 Gy	Seeds	Gradual formation of a number of shoot and fresh weight was declined by increasing irradiation
Bougainvillea	*Bougainvillea glabra*	500 to 2000 Krad	Stem cuttings	Survival rate was 94% at 500 Krad and at 2000 Krad sprouting was delayed
Chrysanthemum	*Dendranthema grandiflora*	5, 10, 20, 30 and 40 Gy	Shoot culture	Flower color was detected by 10 Gy irradiation
Chrysanthemum	*Chrysanthemum moriflium*	10, 15, and 20 Gy	Flower bud	10 and 15 Gy can be used for inducing genetic variability
Chrysanthemum	*Chrysanthemum moriflium*	0.5, 1, 2, and 5 Gy	Shoot culture	Nuclear DNA content was comparatively less at low dose rates

Table 6.
Effect of exposure of gamma irradiation on different economically important flowers and ornamental plants during 2008–2019 [48].

mutant flower and ornamental plants induced by gamma irradiation, as shown in **Table 5** [47]. The effect of exposure to gamma irradiation on different economically important flowers and ornamental plants is summarized in **Table 6** [48].

7. Conclusions

The technology of radiation, especially gamma irradiation is a faster tool and environmentally friendly. Plant biotechnology, both tissue culture and molecular biology, plays an important role in shortening the time of mutation breeding. Roughly, tissue culture is new mutant lines synthesized and molecular biology is an analyzed tool. Most of the studies on mutagenesis in plants are using gamma-ray with changes in some characteristics that disappeared in the parents. For developing new plant varieties applying gamma radiation or chemical mutagen to *in vitro* explants is a useful and worldwide method. The mutants can be selected and propagated to produce numerous plantlets that will be further accepted. Therefore, useful changes can provide improving new varieties, new species, and sometimes new genera. Several types of plants including food and medicinal plants and ornamental plants have been improved by these methods. Thus, radiation-induced mutation breeding is a remarkable method that can lead to genetic variations, resulting in superior mutant cultivars with new and useful traits.

And all of these are rapidly artificial evolution by humans.

Conflict of interest

The authors declare no conflict of interest

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