Retraction Notice

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Comment:
The Editorial Board would like to extend its sincere apologies for any inconvenience this retraction may have caused.

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Increasing Plant Resistance to Unfavorable Conditions and Yields Using Radiation-Induced Growth Simulation

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Abstract

As a predicted result of increasing population worldwide, improvements in the breeding strategies in agriculture are valued as mandatory. The natural resources are limited, and due to the natural disasters like sudden and severe abiotic stress factors, excessive floods, etc., the production capacities are changed per year. In contrast, the yield potential should be significantly increased to cope with this problem. Despite rich genetic diversity, manipulation of the cultivars through alternative techniques such as mutation breeding becomes important. Radiation is proven as an effective method as a unique method to increase the genetic variability of the species. Gamma radiation is the most preferred physical mutagen by plant breeders. Several mutant varieties have been successfully introduced into commercial production by this method. Combinational use of *in vitro* tissue culture and mutation breeding methods makes a significant contribution to improving new crops. Large populations and the target mutations can be easily screened and identified by new methods. Marker-assisted selection and advanced techniques such as microarray, next generation sequencing methods to detect a specific mutant in a large population will help the plant breeders to use ionizing radiation efficiently in breeding programs.

Subject Areas

Crop Science

Keywords

Ionization, Radiation-Induced Simulation, Crop Yield, Plant Resistance, Growth Simulation

1. Introduction

Currently, agriculture faces many challenges associated with global climate change and the gap between food demand and the availability of agricultural land. As a result, there is now a growing understanding that "business as usual" in agriculture will be insufficient to meet these challenges. This emphasizes the need for development of new technologies like radiation-induced growth stimulation, aimed to increase plant resistance to unfavourable conditions and yields. It is well known that low doses of gamma rays can stimulate cell division, growth and development in plants (Cedergreen *et al.*, 2021) [1]. Although a conclusive explanation for the stimulation effects of gamma rays has not become available yet, some research supports a hypothesis that changes in enzyme activities, phytohormonal balance and an increase in the antioxidant capacity of cells are involved in this process. Several studies have shown improvement not only in root and shoot elongation, but also in biomass growth and harvested yield in plants exposed to different kinds of stress (Velini *et al.*, 2020 [2]; Cedergreen *et al.*, 2021 [1]). From an agricultural point of view, stimulatory effects on harvestable plant traits possess the potential for improving crop production and quality. However, high reproducibility of effects is an essential prerequisite for implementing new agricultural practices.

Plant mutation breeding is a core area among modern approaches that is practiced as a part of plant breeding technology. The contribution of plant breeding combined with modern technologies for expansion of crop production toward improved food security and nutrition is recognized worldwide. With increasing population and concomitant decreasing land resources, improvement in crop yields based on fertilizer utilization, and the control of insects, pests, and pathogens need to urgently be implemented and face several challenges (Ahloowalia & Maluszynski, 2021) [3]. In the early twentieth century, plant biologists determined that application of chemical radiation technology could achieve an increased frequency of genetic modifications and efficiency in treated seeds (Oladosu *et al.*, 2021) [4]. Thereafter, a variety of mutagens, such as physical or chemical, were utilized to induce an extensive range of genetic variability that has been prompted and contributed to current plant breeding.

Radiation-induced mutation breeding is a remarkable method that presents superior mutant cultivars in contrast to conventional breeding like selection and crossing which is time-consuming and laborious with limited induced genetic alteration (Beyaz & Yildiz, 2021) [5]. In plant mutation, breeding hinges not only on its effectiveness but also on its efficiency owing to the convenience of physical or chemical mutagens. Mutagenic effectiveness, which is the rate of mutations produced by a mutagen based on the response of a cultivar to increasing doses of the mutagen determines the mutation rate of the destructive effects. Therefore, for experimental purposes, irradiation doses ranging from low to high, approximately at 100 Grays, although, up to a kGy could be used in agricultural business or for varietal preferences. In the last few decades, several

studies have focused on the utilization of radiation in gamma rays with a specific interest in superior cultivars of agricultural crops of economic interest (Jan *et al.*, 2021) [6]. Examples of favorable traits induced after gamma exposure include dwarf or semi-dwarf growth pattern, earlier flowering and maturity, high yielding varieties, and resistance to insect and pathogen infestations. Over the previous fifty years, induced mutation has accounted for enhanced resistance varieties (Kharkwal & Shu, 2021) [7], and the mutant database of the Food and Agricultural Organization lists almost 3246 certified mutagenic plant varieties (Beyaz & Yildiz, 2021) [5]. Ornamental plant mutation breeding has become more successful owing to additional changes in phenotypic characteristics, heterozygous nature, and high mutation frequency producing a large number of new varieties. The main purpose of this article seeks to examine the application of ionizing radiation in mutation breeding.

2. Ionize Radiation, Reactive Oxygen Species (ROS) and Defense Systems of ROS

Ionizing radiation causes biological injury in exposed biological materials. The first target of ionizing radiation is water molecule, which is ubiquitous in any organisms. The cell is composed of ~80% water (Atak *et al.*, 2022) [8]. As a result of excitation and ionization reactions, water molecule (H_2O) and H• and OH radicals are generated. Gamma rays cause to produce free radicals (free radicals like O_2•− and OH• and non-radicals like H_2O_2 and O_2) as known reactive oxygen species (ROS) through direct interactions of radiation with target macromolecules or via products of water radiolysis. The formation of reactive oxygen species (ROS) occurs in the general metabolism of the plant cell. However, such as other environmental stress, radiation lead to increase the formation of ROS in plant cell due to damage of cellular homeostasis and cause progressive oxidative damage and finally cell death. Reactive oxygen species (ROS) control many different processes in plants. Plants has two antioxidant machinery, one of them is anti-oxidative enzymes, including ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR). The other one isnon-enzymatic antioxidants like ascorbic acid (AA), reduced glutathione (GSH), *α*-tocopherol, carotenoids, flavonoids, and the osmolyte proline. ROS depends on ionizing radiation level that causes damage or modification of components in plants, ultimately affecting morphology, physiology, anatomy, and biochemistry of plants. Currently, scientific evidence shows that ROS play an important signaling role in plants and regulate biological activities such as growth, development, and especially response to biotic and abiotic stress factors. ROS can induce injury of cell compartment, but on the other hand, they induce new gene expression in cells. However, Esnault *et al.* (2020) [9] hypothesized that ROS (mainly H_2O_2) can play a secondary role are in signaling process of cell. And after a first stress, plants can be more

tolerant to a new stress synthesis due to secondary metabolites. Moreover, using gamma rays can create a permanent gene expression of anti-oxidative enzymes for scaving "oxidative stress" start from the first generation of plants. And this provides to improve superior plants varieties against biotic and abiotic stress factors.

3. Types of Ionizing Radiation

Ionizing radiation (IR) is categorized by the nature of the particles or electromagnetic waves that create the ionizing effect. These have different ionization mechanisms and may be grouped as directly or indirectly ionizing. The physical properties of ionizing radiation types, namely gamma rays X-rays, UV light, alpha-particles, beta-particles, and neutrons, are different; therefore, their potential usage and bio-applicability to the breeding programs are different.

In the beginning of the twentieth century, ionizing radiation has been begun to induce the mutations. They can be particulate or electromagnetic (EM). Their specific feature is the localized release of large amounts of energy. These have different ionization mechanisms, and they can group as directly or indirectly ionizing. The physical and chemical reactions initiate the biological effects of ionizing radiations. Mostly, X-rays had been used, and later gamma rays and neutrons have been preferred. Two forms of electromagnetic radiation, X-rays or gamma (γ) rays, are widely used in biological systems and most clinical applications. Cobalt-60 and cesium-137 (Cs-137) are the main sources of gamma rays used in biological studies. Cesium-137 is more preferred since its half-life is much longer than cobalt-60. Gamma rays are produced spontaneously, whereas X-rays are produced in an X-ray tube (accelerated electrons hit a tungsten target, and then they are decelerated. The Bremsstrahlung radiation is part of the kinetic energy, belongs to the electrons, and is converted to X-rays). Energy transfer is caused by the interaction, it cannot completely displace an electron, and it produces an excited molecule/atom; whenever the energy of a particle or photon exceeds the ionization grade of a molecule, ionization occurs. Ten electronvolt binding energy for the electrons is determined for biological materials, and higher energetic photons are considered as ionizing radiation, whereas the energies between 2 and 10 eV, which cause excitation, are called as nonionizing. Electrons, protons, α-particles, neutrons, and heavy charged ions are clinically used natural radiation types.

4. Effects of Ionizing Radiations

Ionizing radiation (IR) is known to effect on plants. Their effects are classified as direct and indirect. Stimulatory, intermediate, and detrimental effects on plant growth and development are based on dose of ionizing radiation applied to the plant tissues. The main point is to evaluate the impacts of ionizing radiation at genetic level. The severity of the impacts of radiation is in relation with the species, cultivars, plant age, physiology, and morphology of the plants besides their

genetic organizations.

Ionizing radiation causes structural and functional changes in DNA molecule, which have roles in cellular and systemic levels. The nature of DNA modifications includes base alterations, base substitutions, base deletions, and chromosomal aberrations. These modifications are the reasons of macroscopic phenotyping variations (Oladosu *et al.*, 2021) [4].

Interaction between atoms or molecules and ionizing radiations causes free radical production that damages the cells. Free radical is defined as an atom or group of atoms including an unpaired electron. Water in the cell accumulates energy initially and facilitates the production of reactive radicals, which oxidize and reduce. They have a role in direct and indirect actions of ionizing radiations. In direct action, a secondary electron reacts directly with the target to produce an effect, while in indirect action, free radicals produced via radiolysis of water interact with the target to comprise target radicals.

There are substantial data indicating that the lethal effects of radioactive compounds accumulate in nucleus rather than other parts. Therefore, DNA is the main target as a result of ionizing radiation, and it targets DNA directly or indirectly and leads various alterations. Direct ionization of DNA, reactions with electrons or solvated electrons, reactions with OH or H2O+, and reactions with other radicals can damage cellular DNA. There are some possibilities of DNA damages caused by ionizing radiation. IR and secondarily produced reactive oxygen species can cause changes in deoxyribose ring and structures of bases, DNA-DNA cross-links, and DNA-protein cross-links. Hydroxyl radicals react with bases. The reactive intermediates are produced as a result of this interaction (Hosseinimehr, 2021) [10].

Hydroxyl radicals separate hydrogen atoms from the sugar-phosphate backbone of DNA to form 2-deoxyribose radicals, which cause strong damage by attacking oxygen or thiol groups. Researchers have shown that purine and pyrimidine rings, single-strand breaks (SSBs), and base loss regions are damaged by DNA radiolysis products induced by free radicals. The amount of the yield of the individual products is important and reported to be different than produced during oxidative metabolism. Although free radicals attack on DNA and cause several DNA damages, they have not been thought to lead lethal and mutagenic results. Ionizing radiation-induced base damages are widely studied by *in vitro* studies. It is also reported by several studies that direct and indirect radiation effects may produce identical reactive intermediates. Oxygen is another key molecule that determines the biological effectiveness of the ionizing radiation. Oxygen can easily react with many free radicals. The amount of the radicals presents in deoxyribose or bases; harmful DNA damages occur.

5. Mutation Breeding

In nature, mutations acquired new survival traits to the crops against environmental stresses both biotic and abiotic. Many of these survival traits could be

weakened or totally lost in time. Mutations are sudden changes at the genotype level and cause small and exquisite changes in phenotype, which cannot be detected by advanced molecular techniques. Identification of naturally mutated gene is inconvenient. When the breeders pinpoint the mutated gene, wild-type features have to be reestablished. This task is becoming increasingly infeasible due to long time, more human source, and increase in cost. That's why new breeding strategies were needed to be improved to fortify the crops. To achieve this mission, plant breeders should rebuild in crop plants several specific traits, which have role in survival of the plants under extreme conditions providing the other crop-specific traits such as quality, yield, etc. Phenotyping-based processes of conventional breeding strategies should have moved frombase to a high level of genotype-based breeding methods (Schaart *et al.*, 2021) [11]. New technologies should be legal, economic, and ethical for the breeders and the consumers.

Under such circumstances, inducing mutations are potential applications to produce crops with desired traits and easily selected from the germplasm pool. As described above, radiation can cause several effects on genetic material due to the exposure dose. These effects can be classified in both positive and negative approaches. Beside the detrimental effects of radiation, plant breeders are focused on the effective usage of gamma radiation in breeding programs. Changes in agronomic characters can be transmitted to the next generations. Nuclear techniques are begun to be used in plant breeding mostly for inducing mutations. During the past 60 years, we observed a significant increase in the major crops. Ionizing radiations such as X-rays and gamma rays have been used for improvement of several crops such as wheat, rice, barley, cotton, tobacco, beans, etc. (Ahloowalia & Maluszynski, 2021) [3]. Plant breeders are also combined with this resource with different techniques to increase the efficiency and shorten the time. Induced mutagenesis and combined breeding strategies are effective to improve quantitative and qualitative traits in crops in a much shorter time than the conventional breeding procedure.

Gamma radiation is widely used to induce mutations in breeding studies than chemical mutagens. Ionizing radiation could cause several DNA damages randomly; therefore, several mutations (from point mutation to chromosome aberrations) could be induced. Over 3000 mutant varieties of major crops have been reported to be developed by ionizing radiation (Tanaka *et al.*, 2020) [12]. Mutation rate/mutation frequency is defined as the ratio of mutation per locus and also termed as the number of mutant plant per M2 generation. It changes due to per dose and mutagen. The main point is to determine the best dose for inducing mutants rather than its type.

From past to present, it is concluded that the doses between LD50 and LD30 (doses lead to 50% and 30% lethality) are generally useful in mutation breeding programs. The importance of convenient dose that depends on the radiation intensity and exposure time is gestured by the researchers. The final target is to se-

lect the desired mutants in the second and third generations (M2 and M3). It is effective to select the mutants treated by the mutagens with a high mutation frequency from the M1 population. M1 population consists of heterozygous plants. That means during the treatment one allele is affected by the mutation, and it is impossible to discriminate the recessive mutation in this generation. Therefore, the breeders should sift out the next generations to identify the homozygotes for both dominant and recessive alleles (Oladosu *et al.*, 2021) [4]. M2 population is the first generation that the selection begins. Physical, mechanical, phenotypic, and other methods are used for the selection of the mutants. When the plant breeder finds a mutant line, the next step is the multiplication of the seeds for further field and other studies.

According the 2015 data of Food and Agriculture Organization/International Atomic Energy Agency (FAO/IAEA), over 232 different crops including wheat, rice, sunflower, soybean, tomato, and tobacco were subjected to mutation breeding programs and over 3000 mutant varieties with improved properties in over 70 countries.

Instead of waiting for natural mutations to generate a desired trait, creating a mutation with different tools may promote to the breeding studies. The simplicity and low cost of mutation treatments and gamma radiation became an effective tool to improve new agronomic traits in various crops. It may be evaluated as an alternative to genetically modified plants. The released mutation breeding-derived varieties showed the potential usage of mutation breeding as a flexible and available accession to any crop supplied for desired purposes, and discriminating techniques are successfully combined.

6. *In Vitro* Mutagenesis Applications

Induced mutagenesis is a widely used method to identify and isolate the plant genes in combination with molecular accessions. These kinds of studies supply a clear comprehension into the relation of genes and functions of the genes that have role in growth and development under several conditions (Shirly *et al.*, 2020) [13]. (As shown in **Table 1** below)

7. Selection of Species and Explant Types for *In-Vitro* Culture

Correct choice of the plant species due to economic, commercial production capacity and agricultural importance is the first step of an *in vitro* mutagenesis study. The selection of the plant material is related to the success of the *in vitro* culture. Seed, callus, node, shoot, and root tip cultures are the most commonly preferred plant material for *in vitro* mutagenesis applications. The genotype of a plant has a role in *in-vitro* culture studies. The studies showed that different explants of same plant had different responses to the same radiation dose (Zhou *et al.*, 2022) [14]. Therefore, it is necessary to design an *in vitro* mutagenesis experiment in a proper combination of dose and explant type.

Table 1. Applications of induced mutagenesis for improved features in plant breeding.

Crops	Improved Traits
Barley	Phytate (antinutrient)
Tomato	Resistant to bacterial wilt (*Ralstonia solanacearum*)
Canola	Oil quality improvement
Maize	Resistant to pathogen Striga, acidity, and drought tolerance; improvement of protein quality
Mung bean	Resistant to yellow mosaic virus
Rice	Resistant to blast, yellow mottle virus, bacterial leaf blight and bacterial leaf stripe, semidwarf/dwarf cultivar, lodging resistance, acid sulfate soil tolerance; tolerant to cold and high altitudes, salinity tolerance, early maturity, high-resistant starch in rice for diabetes patients, giant embryos of eight more plant oil, low amylose, low protein)
Soybean	Resistant to Myrothecium leaf spot and yellow mosaic virus, oil quality improvement, oilseed meals that are low in phytic acid desirability, poultry and swine feed
Strawberry	Thick and small leaf, light leaf color, white flesh and long fruit, *Phytophthora cactorum* resistance Salinity tolerance
Sunflower	Oil quality improvement, semidwarf/dwarf cultivars
Wheat	Resistant to stripe rust

8. Determination of Proper Gamma Radiation Dose

The most important subject of *in vitro* mutagenesis is to select the suitable radiation dose to obtain the maximum viability. In the beginning, assessment of the LD50 value is needed to optimize the exact mutation dose. The sensitivity of the plants changes due to the species, cultivars, and current physiological environment. A preliminary dose experiment should be performed to define the appropriate dose. Reduced growth and seedling damages may be seen as traces of the genetically damaged plants after irradiation (Ulukapi *et al.*, 2021) [15].

According to the findings of the preliminary studies done with gamma radiation, it has been reported that there is no linearity between the radiation dose and the variance. Seed, callus, shoot tips, node cultures, and bulblets were frequently used for irradiation of different species. ^{137}Cs and ^{60}Co gamma sources were used to induce mutagenesis at different doses depending on the radiosensitivity of the explants. Atak *et al.* (2022) [8] used 100 - 500 Gy radiation doses produced by ^{137}Cs gamma source for soybean seeds, while Singh and Datta (2022) [16] used ^{60}Co gamma source at different doses ranging between 10 and 100 Gy for *Triticum aestivum* seeds.

Ulukapı *et al.* (2021) [15] also used ^{60}Co gamma source at 80 - 240 Gy radiation doses to induce genetic variability for *Solanum melongena* L. Çelik and

Atak (2021) [17] used 100, 200, 300, and 400 Gy gamma rays by ^{137}Cs to determine the effective radiation dose for breeding studies of two Turkish tobacco varieties. They irradiated the tobacco seeds and selected the salt-tolerant mutants in M3 progeny. Seetohul *et al.* (2022) [18] used 0 - 60 Gy gamma doses of ^{60}Co gamma source to induce mutations for shoot tip explants of Taro plant. Jain (2021) [19] irradiated shoot tip explants of Musa spp. By Cesium-137 at 10 - 50 Gy doses, while Baraka and El-Sammak (2021) [20] used 0.25 - 1 Gy for *Gypsophila paniculata* L. shoot tip explants by ^{60}Co gamma source. Atak *et al.* (2022) [8] used shoot tip explants of Rhododendron varieties to induce mutants at 5 - 50 Gy of gamma rays of ^{137}Cs source. In tissue culture treatments, different synthetic chemicals show similar effects as plant growth regulators which have abilities to induce growth of the tissues as desired.

9. Mutational Genomic Analysis

Mutational genomics is becoming a valuable tool to differentiate the mutants improved via mutation breeding programs. It is also an important tool to understand the molecular basis of the plant stress response based on the data gathered from mutants of model plants and an easy way to determine the genetic similarities and characterize the variations between the mutants at the DNA level. The mutants were identified based on morphological characters, traditionally. The new developments in DNA technologies give opportunity to the plant breeders to make it quick and definite (Fangue-Yapseu *et al.*, 2021) [21].

Molecular markers are widely used to differentiate the genetic differences between the mutant and the mother plants through characterizing the variations at DNA level. High-throughput genomic platforms such as random amplified DNA polymorphism (RAPD), cDNA-amplified fragment length polymorphism (AFLP) (Celick *et al.*, 2022), single-strand conformational polymorphism (SSCP), microarray, differential display, targeting induced local lesions in genome (TILLING) and high-resolution melt (HRM) analysis allow rapid and in-depth global analysis of mutational variations.

Among these methods RAPD, inter simple sequence repeat (ISSR), and AFLP have been frequently used in genomic classification of the mutants. RAPD is an inexpressive and a rapid method to use in many fields of biotechnology. There is no need for genome information. It has been widely used to determine the genetic diversity in mutation breeding programs of many plants. RAPD is an efficient method to detect DNA alteration via using random primers. It has been started to use in earlier studies of genetic variabilities obtained by radiation treatments in Chrysanthemum, soybean, sugarcane, sunflower, groundnut, tobacco, potato, Rhododendron (Atak *et al.*, 2022) [8]. ISSR method is another molecular marker method widely used in plant biotechnology applications. It is also easy to apply more informative than RAPD, reliable, and inexpensive. ISSR primers are designed by using microsatellite sequences to amplify the genomic regions flanked by microsatellite repeats. By using one primer, it is possible to

amplify multiple fragments as a result of ISSR analysis. The information obtained from ISSR analysis is more reliable than RAPD to provide supplementary data of the genetic variations of the mutants from the non-overlapping genome regions.

Xi *et al.* (2021) [22] reported an *in vitro* mutagenesis protocol for *Lilium longiflorum* Thunb. cv. White fox. They used 0, 0.5, 1.0, 1.5, 2.0, and 2.5 Gy gamma rays to observe the effects of radiation on adventitious bud formation from bulblet-scale thin cell layers. 1.0 Gy was determined as the most effective dose due to survival rate of the bulblet-scale thin layers. They also evaluated the morphological mutants using ISSR DNA fingerprinting method. Sianipar *et al.* (2020) [23] used RAPD method to detect the genetic variability between the mutant plantlets improved from gamma-irradiated rodent tuber calli. They obtained 69 fragments from 11 mutant plantlets by using 10 RAPD primers.

Barakat and El-Sammak (2021) [20] irradiated shoot tips and lateral buds of *G. paniculata* with four different gamma radiation doses between 0.25 and 1 Gy. They detected the genetic polymorphisms among the mutants by RAPD analysis. They obtained 105 different amplification products from 10 random primers. RAPD is evaluated as an efficient molecular marker technique to detect the variations. Atak *et al.* (2022) [8] used RAPD method to show the genetic similarities of the Rhododendron mutants improved via gamma irradiation. They used 0 - 50 Gy gamma radiation doses to improve the shoot and root regeneration rates of Rhododendron plants. RAPD detected higher genetic variability among the Rhododendron mutants. Yaycılı and Alikamanoğlu (2021) [24] observed 89.66% polymorphism rate with six primers among the mutant potato plants, which were improved as salt tolerant via gamma radiation treatment.

Kaul *et al.* (2021) [25] used *in vitro* mutagenesis in Chrysanthemum cv. Snow Ball by irradiation of the *in vitro* shoots, and genetic polymorphisms among the mutants and the control plants were assessed by RAPD. They reported that 10 Gy gamma irradiation was found as the most effective dose to induce genetic variation in morphological traits, and they observed 100% polymorphism among the mutants. Gamma radiation-induced salt-tolerant oriental tobacco mutants were improved by Çelik and Atak (2021) [17]. Salt tolerance of the mutants was controlled by the callus induction in the presence of high salt concentration. The genetic similarities of the mutants were determined by RAPD analysis. The relationships between the salt-tolerant mutants and controlled tobacco varieties were shown in Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram. Sen and Alikamanoğlu (2021) [26] used ISSR method to differentiate the drought-tolerant sugar beet mutant improved via irradiation of shoot tip explants by gamma radiation. They obtained 91 polymorphic bands of 106 PCR fragments with 19 inter simple sequence repeat (ISSR) primers.

Perera *et al.* (2020) [27] applied *in vitro* mutagenesis treatment to an important energy crop giant miscanthus (Mischanthus × giganteus) to induce variation in cultivar Freedom. ISSR markers were used to determine the variations in

the mutant plants. The putative mutants were selected due to the results of molecular marker analysis to use for further bioenergy researches. Wu *et al.* (2021) [28] used ISSR analysis to show the genetic similarities between the mutants. For this reason, they used 60 ISSR primers, and 60 polymorphic bands of 392 were evaluated to have information on the molecular level of mutation breeding. Atak *et al.* (2022) [8] used ISSR marker method (with 61 ISSR primers) to define the genetic variation among the 8 salt-tolerant mutant soybeans obtained from *in vitro* mutagenesis treatment by using ^{137}Cs gamma source.

Single-strand conformational polymorphism (SSCP) is another strength method to identify the variations between the mutant and mother plants in amplified DNA samples. It is widely used to determine the genetic mutations in several organisms (Maluszynski *et al.*, 2021). It is also an effective method to find a potential genetic marker which is in relation with a desired trait to use in selection studies of agricultural populations. Irradiation of the plant tissues can cause mutation between the allelic gene copies [single-nucleotide polymorphism (SNP)]. SSCP is an efficient method to detect these polymorphisms. It is possible to detect relations between SSCP polymorphisms and quantitative traits.

These methods can only be able to detect the genetic variations of the mutants in accordance with the mother plants. There are a number of methods to screen the causal mutation at a desirable phenotype. Molecular markers that are in relation with the mutation are known to be able to segregate in the next progenies (Al-Rumaih & Al-Rumaih, 2022). The main point is to make the functional analysis of the mutant genes that have role in acquiring the new desired characters. To identify a mutant, the number of the genes controlling that specific phenotypic character is deterministic.

In a mutation breeding program, identification of differentially expressed genes, the biological processes they have role in, or the metabolic pathways of interest should be carried out through modern genomics and system biology. To achieve this, there are specific tools to discriminate with the use of next-generation molecular techniques. In microarray systems, it is available to detect the gene expressional differences between the mutants and control plants. Thousands of spots on a microarray chip containing a few million copies of identical DNA molecules buried on each spot are related to each gene of a plant genome. If it is a targeted mutation, it is possible to show the expressional differences between them by microarray technique. In general, spontaneous mutations cannot be detected at microarray systems. Sequencing methods are more efficient in the meanwhile. Mutant plants can now easily sequence by next-generation sequencing (NGS) techniques to define the mutations.

10. Discussion

The increasing importance of plant breeding studies in correlation with biotechnology and molecular genetics attempts to meet the requirements of increasing population for food and crop plants. Therefore, mutation breeding treat-

ments have become more frequent and alternative to classical breeding and genetically modified plants. The main aim is to combine several features of many plants in one super plant. *In vitro* mutagenesis has become an efficient tool for this purpose. Plant breeders are focusing on crop improvement techniques to improve genetic variations of useful traits by using next-generation molecular methods.

11. Conclusions

Using these advanced genomic techniques, new molecular mechanisms and new genes can be potentially identified by the plant breeders as a result of *in vitro* mutagenesis treatments.

To gain more data, additional needs of various comparative and descriptive experiments can be upgraded to acquire more specific points to build the relations among the regulatory mechanisms. Therefore, the recent progress in mutation breeding studies concerning new technologies is quite important to contribute new advancements to plant breeding programs.

Conflicts of Interest

The author declares no conflicts of interest.

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