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Breeding crops for climate resilience by mutation

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Abstract
Agricultural breeding strategies must be improved as a result of the predicted rise in the global population and unpredictable climate change. Breeders face enormous challenges in trying to feed the steadily growing human population amidst the dangers posed by these unfavorable climate changes. Heightening germplasm diversity through mutation is indispensable in contemporary and classical radiation breeding because it has the latent to produce random mutations in the entire genome. This is true even though avant-garde technologies such as gene editing have made it a reality to breed varieties by editing one or more specific target genes**.** This technique has been successfully used to introduce several mutant varieties for industrial production. This review discusses the current state of conventional and particle radiation mutation breeding and illustrates the molecular mechanisms of radiation-induced mutations. This review also examines the prospects of radiation mutation breeding, which will help expand our understanding of its exploration for human benefits against unfavorable weather patterns.

Keywords: Mutation breeding, Mutagenesis, Biotic and abiotic stress, classical radiation, particle radiation, climate

1. Introduction
The most fundamental assurance for human survival on Earth is provided by crops, and domestication is crucial for transforming wild plants into cultivated crops by long-term screening for desirable traits caused by gene mutations [1, 2]. Nevertheless, the shift in global climate change and its impact, coupled with the ever-growing human population, cannot be overlooked [3]. These threats constantly call for the immediate development of crops that are highly resilient and adaptable to meet global food needs. A significant problem is increasing agricultural yields to ensure food security. Climate change (Rising temperatures and erratic rainfall), which frequently reduces crop productivity, is a barrier to this [4]. Another is the need to produce more food and crops for bioenergy while reducing the carbon production costs [5]. Therefore, there is a pressing need for new, high-yielding cultivars with enhanced nutrient and water use efficiency management [6-9]. This highlights the requirement for the creation of novel technologies, such as radiation-induced growth stimulation, aimed at enhancing plant yields and resistance to unfavorable conditions. The practice of creating new biological cultivars through chemical or radiation mutagenesis is known as mutation breeding. Chemical mutagenesis, which primarily causes point mutations in genes, is the biochemical reaction between chemical agents and genetic material. The environmental optimization and biological safety of chemical mutagenesis need to be improved despite their effectiveness. In contrast, radiation mutagenesis results in more complex genetic mutations and more advantageous mutant phenotypes. Space radiation, particle, and classical radiation mutation breeding are the three main categories of radiation mutation breeding. X-ray and Gamma-ray applications are the two main types of traditional radiation mutation-breeding techniques. Classical radiation mutation breeding is a widely used technique that is effective against crop variation. This technique primarily refers to the process of using different rays to induce a significant number of genomic mutations and to accelerate the production of mutant traits through direct or indirect energy deposition onto DNA. Using this method, it is possible to induce desirable traits that are either not expressed in nature or have been lost through evolution. In addition, the use of traditional radiation mutation technology has resulted in the development of numerous new varieties that are frequently used in farming [10]. The rate of mutations caused by a mutagen based on how a cultivar reacts to progressively higher doses of the mutagen determines the mutation rate of the destructive effects. Accelerated particles, such as heavy ions or protons, are typically used in particle mutation breeding.

They have distinctive physical characteristics such as depth-dose distribution, a variety of radiation parameters, and a complex track structure. Because they produce excellent biological mutagenic effects at relatively low radiation doses, accelerated particles have been regarded as potent mutagens for crop breeding [11]. Particle radiation mutagenesis is notable for its ability to create novel cultivars with desirable characteristics without adversely affecting other phenotypes [12].

With a particular interest in better cultivars of crops of economic interest, several studies have focused on the use of gamma-ray radiation over the last few decades [13]. Dwarf or semi-dwarf growth patterns, earlier flowering and maturation, high-yielding varieties, and resistance to insect and pathogen infestations are some examples of advantageous traits induced after gamma exposure. The Food and Agricultural Organization's mutant database contains information on nearly 3,246 plant varieties that have been certified as mutagenic [12], and induced mutations have been attributed to enhanced resistant varieties [14]. Owing to additional changes in phenotypic characteristics, heterozygous nature, and high mutation frequency, ornamental plant mutation breeding has become more successful, leading to the creation of numerous new varieties. It is well known that plants can be stimulated to divide their cells, grow, and develop when exposed to low doses of gamma radiation [1]. The hypothesis that changes in enzyme activities, phytohormonal balance, and an increase in the antioxidant capacity of cells are involved in this process is supported by some studies, despite the lack of a conclusive explanation for the stimulatory effects of gamma rays. This review discusses the current state of conventional radiation mutation breeding to elucidate the molecular mechanisms underlying radiation-induced mutations. This review also examines the prospects of radiation mutation breeding, which will help expand our understanding of its exploration for human benefits.

2. The History and Mechanism of Conventional Radiation Mutation

The origins of plant mutations can be traced back to 300 BC through documented accounts of mutated crops in China [15, 16]. Since then, new crop varieties have been cultivated in large parts using radiation mutation breeding. Mutations have been recognized as a significant mechanism for generating variation since the late 1800s when Hugo de Vries conducted experiments aimed at "rediscovering" Mendel's laws of inheritance [17]. The variability in question was considered to be the result of heritable changes that are governed by mechanisms that significantly differ from those of segregation and recombination. He depicted the event as rapid modifications in organisms of a hereditary nature, fostering considerable impacts on the phenotypic outcome of the organism. He subsequently introduced the term mutation and posited a compounded concept concerning the emergence of abrupt, drastic alterations (Termed 'leaps') in preexisting traits, ultimately giving rise to the formation of a novel species and variation. The mutagenic potential of radiation was first postulated by Muller in 1928, who provided compelling evidence that X-ray exposure can induce genetic mutations [16]. Following Stadler's initial publications on the induction of mutations in maize and barley through radiation exposure, this method has garnered substantial usage as a tool for advancing the development of novel high-yielding crop cultivars and generating genetic resources [18, 19]. In 1938, a study conducted by Nilsson-Ehle and Gustafson involved examining the effects of X-rays and UV light on barley resulted in the identification of various mutants. These mutants were classified based on their carotenoid and chlorophyll concentrations and distribution within the leaf blade into Albina, Xantha, Alboviridis, Viridis, Tigrina, Striata and Maculate [20]. The quality of several mutated lines was identified as highly advantageous for prospective application in the field of agriculture due to their displayed modifications, such as grain yield, straw rigidity, straw size and multiplicative potential. Additionally, they demonstrated alterations in spike solidity, kernel maturation and coloration [21]. Subsequently, the varietals '*Trebi*' and 'Moister' of the barley plant were subjected to the radiation stemming from the initial aerial atomic explosion at Bikini Atoll in 1946 [22]. Radiation mutation breeding stands out among other breeding methods such as cross-breeding and chemical mutagenesis due to its exceptional advantages. These include a considerably broad mutation spectrum and high efficiency in inducing mutations [23]. Compared to chemical mutagens, gamma radiation is more widely used to induce mutations in breeding studies. Ionizing radiation could cause several random DNA damages therefore, several mutations ranging from point mutations to chromosome aberrations could be induced. Over 3000 mutant varieties of major crops have been reported to have been developed by ionizing radiation [11]. The mutation rate or mutation frequency is defined as the ratio of mutations per locus and is also termed as the number of mutant plants per M2 generation. It changes due to dose and mutagen. The main point is to determine the best dose for inducing mutants rather than their type. From past to present, it has been concluded that doses between LD50 and LD30 (doses leading to 50% and 30% lethality) are generally useful in mutation breeding programs. The importance of a convenient dose that depends on the radiation intensity and exposure time is emphasized by the researchers. The final target is to select the desired mutants in the second and third generations (M2 and M3). It is effective to select mutants treated by mutagens with a high mutation frequency from the M1 population. The M1 population consists of heterozygous plants. This means that during the treatment, one allele is affected by the mutation and it is impossible to discriminate the recessive mutation in this generation. Therefore, breeders should sift out to the next generations to identify homozygotes for both dominant and recessive alleles [24]. The M2 population is the first generation in which the selection begins. Physical, mechanical, phenotypic and other methods are used for the selection of mutants. When the plant breeder finds a mutant line, the next step is the multiplication of the seeds for further field and other studies.

3. Types of radiation mutagenic sources

Positive mutations are modifications to the genotypic structure that boost a species' diversity and help it adapt to different selection factors [25]. The agents that give rise to synthetic variations are termed mutagens. They are generally divided into chemical and physical mutagens [25, 26]. In conventional agricultural practice, the induction of mutations in crops is achieved by exposing the planting materials to mutagenic agents of a chemical and physical nature. Various types of planting materials can be utilized in the process of mutagenesis, e.g., entire plants, bulbs, tubers, cuttings, seeds, pollen, rhizomes, corms, seedlings and cultured cells maintained *in vitro* are typically considered. However, seed is the predominant botanical material utilized [27]. Additionally, the efficiency of inducing mutations in vegetatively

propagated plants has undergone a positive transformation as contemporary scientists exploit the unique attributes of totipotency (The ability of a single cell to divide and produce all of the differentiated cells in an organism to regenerate into whole plants), employing various forms of *in vitro* cultured plant tissues, including individual cells [28, 29]. The induction of point mutations is typically achieved through the utilization of chemical mutagens, while physical mutagens can cause extensive lesions, most notably chromosomal rearrangements or abbreviations [30].

3.1 Physical Mutagen

3.1.1 Ionizing radiation

Physical mutagens, primarily ionizing radiation, have been extensively utilized in inducing genetic mutations over the past 80 years and more than 70% of the mutant types have been developed from physical mutagenesis [31, 32]. Ionizing radiation is categorized according to the nature of the particles or electromagnetic waves that create the ionizing effect. Ionizing radiation has different ionization mechanisms and could be accordingly grouped into directly or indirectly ionizing [33]. Since the beginning of the twentieth century, ionizing radiation has been used to induce mutations [16]. Its specific feature is the localized release of large amounts of energy. Ionizing radiation has the ability to cause changes, in living cells through means, including damaging DNA and disrupting normal cellular processes [33-36]. This category of radiation includes ultraviolet (UV) light that can ionize as high energy particles like X rays and gamma (γ) rays. We often use X rays and gamma rays in research and medical settings due to their availability and ease of use. When it comes to applications Cs 137 isotope is often preferred over Co 60 because it has a half-life. It's important to note that gamma rays and X rays are generated differently-X rays require the acceleration and deceleration of electrons, in an X ray tube while gamma rays occur spontaneously. 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 or atom. Ionization occurs when the energy of a particle or photon exceeds the ionization grade of a molecule. Ten electron volt binding energy for the electrons is determined for biological materials and higher energetic photons are considered ionizing radiation, whereas energies between 2 and 10 eV, which cause excitation, are called nonionizing. Electrons, protons, α-particles, neutrons and heavily charged ions are clinically used natural radiation types. These resources are optimally utilized for materials, particularly those in a dry state, such as seeds [37]. Multiple forms of neutrons were thoroughly examined during the 1960s and 1970s regarding their potential capabilities in mutagenesis. The implementation of neutrons as an effective means of inducing mutagenesis has been demonstrated, particularly in the context of producing massive deletions of DNA fragments. Nevertheless, the application of neutrons in this manner is subjected to certain limitations [17]. Ionizing emission has the propensity to penetrate deeper into the cellular tissue, thereby exhibiting the capacity to induce a significant number of alterations within the chemical composition of the affected tissue [38]. One of the primary benefits of employing physical mutagenesis, as opposed to chemical mutagenesis, is the superior precision and reproducibility that it affords, with particular emphasis on gamma rays, which exhibit homogenous tissue penetration. Over the course of the last twenty years, the use of ion beams, whether through implantation or irradiation, has emerged as a novel physical mutagenesis approach, superseding the more prevalent employment of gamma rays, X-rays and neutrons [39-41]. The described phenomenon comprises multiple particles traversing a trajectory, differing in their mass, ranging from a basic proton to a uranium atom. These entities are created through the utilization of particle accelerators. As a result of positively charged ion acceleration, a high velocity is achieved, reaching approximately 20-80% of the light speed. This leads to the formation of high linear energy transfer (LET) radiation. The impact of LET radiation on biological systems is notable due to its ability to induce various significant effects, including chromosomal aberrations and lethality when compared to other forms of radiation utilized in physical mutagenesis. Comparative analysis between the repair of DNA double-strand damage caused by ion beams and gamma rays reveals that ion beams induce a greater incapacity for repair, owing to the elimination of fragments of various sizes within the DNA structure [42].

Recently, the phenomenon of mutation induction has been investigated in plant materials by conducting experiments in the outer space to better understand its intricacies. Speculation has arisen regarding the distinct conditions present during space flight, encompassing factors such as cosmic radiation, microgravity and a meager geomagnetic field. This phrase denotes the existence of viable factors capable of inducing genetic alterations. Currently, there is a dearth of information regarding the fundamental genetics underlying the phenomenon of aerospace mutagenesis [17].

Gamma-ray mutagenesis has been the most commonly used technique for generating plant mutations since the 1960s. A total of 1,600 of the 3,281 mutant cultivars that are officially listed in the FAO/IAEA mutant variety database (http://mvgs.iaea.org) were produced by gamma irradiation. Moreover, the majority of the variations in mutants in Asia were generated using gamma radiation [43]. Gamma ray beams are ionizing radiations that could directly dissect the chemical bonds of cells in the matter by generating or trapping electrons (A process known as ionization) [44]. The linear energy transfer of gamma rays is 0.2 keV·μm–1 as compared to others, either by choosing ion beams or manipulating velocity [45].

3.1.2 Nonionizing radiation

In an effort to elucidate the genomic effects of ultraviolet (UV) radiation on cellular progeny, researchers exposed polar cap cells of fruit fly eggs to controlled levels of UV irradiation. Subsequent studies confirmed the findings through investigation into multiple organism models, revealing that the mutational potential of UV extends beyond this single model system. Upon examination of the germinal tissue from these organisms, evidence of covalently linked pyrimidine structures, known as Cyclobutane Pyrimidine Dimers (CPDs), emerged [17, 46]. Notably, compared to ionizing radiation, UV light exhibits restricted penetration capabilities within biological tissues due to its limited depth of field. As the dominant source of genotoxicity across the globe, UV radiation is well established as the primary contributor to genomic damage arising from solar radiation exposure [47]. This phenomenon encompasses the entirety of the electromagnetic radiation spectrum spanning 100-400 nanometers, with the UV region consisting of three subcategories - short UV-C (100-280 nm), intermediate UV-B (280-315 nm), and long wavelength UV-A (315-400 nm).

Ultraviolet radiation inflicts significant damage upon the genetic material of living organisms, producing diverse forms of DNA lesions such as CPDs and 6-4 photoproducts (6-4 PPs) [48-50]. The distribution and incidence of certain types of DNA lesions in eukaryotic genomes are determined by factors such as sequence composition and chromatin structure following exposure to ultraviolet (UV) radiation. Specifically, cyclobutane pyrimidine dimers (CPDs), which account for up to 90% of all pyrimidine dimers generated in plant cells after UV exposure, have the potential to disrupt transcriptional complexes and alter gene expression patterns [51]. The presence of CPDs possesses the capability to obstruct the transcribing complexes, thereby resulting in a complete modification of the relative expression pattern of genes [52]. During DNA replication, dimers can be effectively bypassed by specialized translesion. In addition, during DNA replication, dimers can be bypassed by specialized translesion DNA polymerases, allowing cells to better withstand UV damage [53-55]. UV radiation has the potential to cause oxidative DNA damage through the mediation of both endogenous and exogenous photosensitizers, leading to the generation of free radicals upon activation. This process is primarily driven by endogenous photosensitizers, however, is not solely limited to them. The genotoxic effects of oxidative DNA damage were unambiguously demonstrated in mammalian cells [56]. While rare occurrences of UV-induced oxidative DNA lesions in plants have been reported, it is possible that these lesions, which are corrected through an error-prone excision repair pathway, contribute to the UV-mediated mutagenesis and genomic instability seen in plant cells [57]. Photoreactivation plays a key role in repairing pyrimidine dimers in plants, suggesting that oxidative DNA damage, which is handled via the less accurate excision repair process, might also participate in the observed UV-induced genetic changes and genomic instability in plant cells.

Table 1: Examples of commonly used physical mutagens [58, 59]

Mutagen	Source	Characteristics	Hazard
X-rays	X-ray Machine	Electromagnetic radiation; penetrates tissues from a few millimeters to many centimeters	Dangerous, penetrating
Gamma rays	Radioisotopes and nuclear reaction	Electromagnetic radiation produced by radioisotopes and nuclear reactors; very penetrating into tissues; sources are 60Co (Cobalt-60) and 137Cs (Caesium-137)	Dangerous, very penetrating
Neutrons	Nuclear reactors or accelerators	Electromagnetic radiation produced by radioisotopes and nuclear reactors; very penetrating into tissues; sources are 60Co (Cobalt-60) and 137Cs (Caesium-137)	Very hazardous
Beta particles	Radioactive isotopes or accelerators	Produced in particle accelerators or from radioisotopes; are electrons; ionize; shallowly penetrating; sources include 32P and 14C	May be dangerous
Alpha particles	Radioisotopes	Derived from radioisotopes; a helium nucleus capable of heavy ionization; very shallowly penetrating	Radioisotopes
Protons	Nuclear reactors or accelerators	Produced in nuclear reactors and accelerators; derived from hydrogen nucleus; penetrate tissues up to several centimeters	Very dangerous
Ion beam	Particle accelerators	Produced positively charged ions are accelerated at a high speed (approximately 20%–80% of the speed)	Dangerous

3.2 Chemical Mutagenesis

The impact of chemical mutagens on plant materials is commonly perceived as less severe in nature [37]. A notable benefit of chemical mutagenic agents lies in their applicability without requiring complex equipment or facilities. In general, compared to physical mutagens, chemical mutagens exhibit a higher ratio of mutational occurrences to undesirable modifications [37]. Chemical mutagens are known to possess a proclivity toward being carcinogenic. Despite the extensive assortment of mutagenic agents available, only a limited proportion has been investigated on plants [60]. Based on an analysis of data, from the International Atomic Energy Agencys (IAEA) database on mutant plant varieties it has been discovered that, than 80% of these variants were created using chemical mutagens called alkylating agents [61, 62]. Among these chemical mutagens, a group of three substances. Ethyl methane sulfonate (EMS) 1 methyl 1 nitrosourea and 1 ethyl 1 nitrosourea collectively contribute to 64% of the examined plant varieties [60]. Ethyl methane sulfonate (EMS; $CH_3SO_2OC_2H_5$) has been shown to be a truly compelling and productive mutagen and is the foremost well-known chemical mutagen [63]. It is a colorless fluid compound with an atomic weight of 124 and is 8% dissolvable in water. EMS has a place together with other alkylating agents. These compounds possess one or more alkyl groups that exhibit reactivity and can effectively undergo transference onto other molecules in areas of increased electron density [64, 65]. Based on the quantity of their functional groups, alkylating agents can be categorized as monofunctional, bifunctional, or polyfunctional. Alkylating agents possessing multiple functional groups, bifunctional and polyfunctional, typically exhibit greater levels of toxicity than monofunctional agents. EMS functions as a monofunctional alkylating agent. Alkylating agents exhibit high levels of reactivity, even when exposed to water. The velocity of hydrolysis is commonly evaluated through the use of the half-life metric, which represents the interval required for the decomposition of 50% of the original quantity of the alkylating composition [65]. The decay kinetics of the EMS chemical compound in water at a temperature of 20°C and a pH of 7. 0 exhibits a half-life of 93 h, while at a temperature of 30 °C, its decay half-life is shortened to 26 h. The chemical reaction between EMS and water is observed as follows:

$$CH_3SO_2OC_2H_5 + H_2O \rightarrow CH_3SO_2OH + C_2H_5C_2H_5OH$$

In a more formal and academic style, through the process of hydrolysis, the ester $CH_3SO_2OC_2H_5$ reacts with water (H_2O) to yield methanesulfonic acid (CH_3SO_2OH) and ethanol ($C_2H_5C_2H_5OH$). Alkylating compounds are also categorized as radiomimetic agents because of the effects they produce, which are similar to those of ionizing radiation [66]. The following reactions resulted from alkylating DNA [67]. The formation of unstable triesters causes the release of the alkyl group and disruption of DNA replication. The DNA backbone can sometimes be broken when the phosphate triesters are hydrolyzed between sugar and phosphate. The ability of point mutations caused by chemical mutagens to produce both gain- and loss-of-function phenotypes, such as tolerance to the herbicides glyphosate and sulfonylurea demonstrated in the

legume *Medicago truncatula*, is a clear advantage of these mutations [68, 69]. The efficiency of a mutagenesis is influenced by the mutagen concentration, the treatment duration and the experimental temperature [66]. Using new batches of the chemical (s), that have been properly stored, as chemical mutagens are very reactive.

Table 2: Examples of well-known chemical mutagens [66]

Mutagens	Example	Mode of Action
Alkylating agents	1-methyl-1-nitrosourea (MNU); 1-ethyl-1-nitrosourea (ENU); methyl methanesulfonate (MMS); ethyl methanesulfonate (EMS); dimethyl sulfate (DMS); diethyl sulfate (DES); 1-methyl-2-nitro-1-nitrosoguanidine (MNNG); 1-ethyl-2-nitro-1-nitrosoguanidine (ENNG); N,N-dimethylnitrous amide (NDMA); N,N-diethylnitrous amide (NDEA)	React with bases and add methyl or ethyl groups and, depending on the affected atom, the alkylated base may then degrade to yield an abasic site, which is mutagenic and recombinogenic, or mispair to result in mutations upon DNA replication
Azide	Sodium azide	Same as alkylating agents.
Hydroxylamine	Hydroxylamine	Same as alkylating agents
Antibiotics	Actinomycin D; mitomycin C; azaserine; streptonigrin	Chromosomal aberrations are also reported to cause cytoplasmic male sterility.
Nitrous acid	Nitrous acid	Acts through deamination, the replacement of cytosine by uracil, which can pair with adenine and thus through subsequent cycles of replication lead to transitions.
Acridines	Acridine orange	Intercalate between DNA bases thereby distorting the DNA double helix and the DNA polymerase in turn recognizes this stretch as an additional base and inserts an extra base opposite this stretched (intercalated) molecule. This results in frame shifts, i.e., an alteration of the reading frame.
Base analogues	5-bromouracil (5-BU); maleic hydrazide; 5-bromodeoxyuridine; 2-aminopurine (2AP)	Incorporate into DNA in place of the normal bases during DNA replication thereby causing transitions (purine to purine or pyrimidine to pyrimidine); and tautomerization (existing in two forms which interconvert into each other, e.g., guanine can exist in keto or enol forms).

4. Radiation sensitivity of plants

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

Ionizing radiation causes biological injury to exposed biological materials. The first target of ionizing radiation is water molecules, which are ubiquitous in all organisms (water constitutes 80% of the living celss) [70]. As a result of excitation and ionization reactions, water molecules (H_2O) and H• and OH radicals are generated. Gamma rays cause free radicals (free radicals such as O2• and OH• and nonradicals such as H_2O_2 and 1O2) as known reactive oxygen species (ROS) through direct interactions of radiation with target macromolecules or via products of water radiolysis. The formation of ROS occurs in the general metabolism of the plant cell. However, as with other environmental stresses, radiation leads to an increase in the formation of ROS in plant cells due to the damage of cellular homeostasis, which causes progressive oxidative damage and finally cell death. Reactive oxygen species (ROS) control many different processes in plants. Plants possess dual antioxidant systems to counteract oxidative stress arising from exposure to ionizing radiation. Plants have mechanisms to protect themselves against damage caused by ionizing radiation. One set of defense mechanisms involves enzymes, like peroxidase (APX) glutathione reductase (GR) superoxide dismutase (SOD) catalase (CAT) guaiacol peroxidase (GPX) monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR). In addition to these enzymes plants also possess enzyme antioxidants such as ascorbic acid (AA) reduced glutathione (GSH) α tocopherol carotenoids, flavonoids and the osmolyte proline. These antioxidants play a role in neutralizing Reactive Oxygen Species (ROS) generated by ionizing radiation. ROS can cause harm to plant cells by altering their structure and leading to changes in morphology, physiology, anatomy and biochemistry. Therefore these antioxidant defenses are vital, for maintaining the health and proper functioning of plants when faced with stress. Currently, scientific evidence shows that ROS plays an important signaling role in plants and regulates biological activities such as growth, development and especially responses to biotic and abiotic stresses. ROS can induce injury to cell compartments, they also induce new gene expression in cells. However, it was hypothesized that ROS (mainly H_2O_2) can play a secondary role in the signaling process of cells [71]. After the first stress, plants can be more tolerant to a new stress synthesis due to the secondary metabolites. Moreover, using gamma rays can create a permanent gene expression of antioxidative enzymes to reduce “oxidative stress” starting in the first generation of plants. This provides superior plant varieties against biotic and abiotic stresses. Ionizing radiation has a known effect on plants and the radiosensitivity of plants can be actualized in different ways. Their effects are classified as direct and indirect, i.e., stimulatory, intermediate and detrimental effects, on plant growth and development are based on the dose of ionizing radiation applied to the plant tissues. The main point is to evaluate the impacts of ionizing radiation at the genetic level. The severity of the impacts of radiation is related to the species, cultivars and plant age, physiology and morphology besides its genetic organization. Plants' radio sensitivities, as rated by exposure to irradiation, have revealed that radio sensitivity can vary by as much as 500-fold between species. The gap increases by at least 5000 times if the algae are taken into account [72]. The meristematic areas must be extremely radiosensitive since growth suppression is the first noticeable side effect of radiation. The sensitivity of *Vicia faba* (broad bean) has been effectively demonstrated through the use of growth rate curves of roots that have undergone X-ray treatments following a brief non-lethal treatment [73]. The elements affecting the radiosensitivity of vegetative development including,

environment, chromosome number, material quality and dosage are the foundation for predicting potential radiation impacts on vegetation. These considerations appear to be applicable to the problem of radio sensitivity in general. Plant radiosensitivity may also be modified by differences in chemical composition; for example, greater ascorbic acid levels may be associated with increased tolerance [74]. Ionizing radiation alters the DNA molecule's composition and functioning, which affect it on the cellular and systemic level. Base adjustments, bases replacement and deletion and chromosomal defects are among the types of DNA modifications [75, 76]. Ionizing radiation interacts with atoms or molecules resulting in free radicals, which harm the cells. An atom or group of atoms with an unpaired electron is known as a free radical. Initially, water in the cell stores energy and aids in the creation of reactive radicals that oxidize and decline. Both the direct and indirect effects of ionizing radiation are influenced by them. In a direct reaction, a secondary electron interacts directly with the target to cause a reaction, whereas in the indirect reactions, target radicals are created when free radicals are formed during the radioactive breakdown of water linking with the target [77]. Substantial data indicate that the lethal effects of radioactive compounds accumulate in the nucleus rather than other parts. Therefore, DNA is the main direct or indirect target because of ionizing radiation and leading to various alterations. Direct ionization of DNA including, reactions with electrons or solvated electrons, reactions with OH or H_2O+ and reactions with other radicals can damage cellular DNA. DNA content boost with increasing nuclear or chromosome size, suggesting a relationship between total DNA per diploid nucleus and sensitivity. The average DNA value per chromosome correlates better with the dose required for potent growth inhibition than the average DNA value per diploid nucleus. DNA values can be used to predict radiosensitivity. High values indicate high sensitivity and low values indicate high tolerance [72]. There are some possibilities for DNA damage caused by ionizing radiation. Ionizing radiation and secondarily produced reactive oxygen species can cause changes in the 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 [78]. 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 yield of the individual products is important and reported to be different than that produced during oxidative metabolism. Although free radicals attack DNA and cause DNA damage, they have not been thought to lead to lethal and mutagenic results. Ionizing radiation-induced base damage has been widely *in vitro* studied. Several studies have also reported that direct and indirect radiation effects may produce identical reactive intermediates. Oxygen is another key molecule that determines the biological effectiveness of ionizing radiation. Oxygen can easily react with many free radicals. The amount of radicals presents in deoxyribose or bases; harmful DNA damage occurs.

4.2 Direct and Indirect Effects of Ionizing Radiation in Plants

4.2.1 Direct Effects

DNA can be degraded when ionizing radiation energy is directly deposited into it. Double-stranded breaks (DSBs) are just one type of DNA damage that may be caused by several chemical and physical processes, but ionizing radiation is one of the few [79]. The emergence of a double-stranded molecule as genetic material where a second strand serves as a template for repairing broken bases or nucleotides is likely favored by factors that produce damage on a single strand [80]. Chromosomes with many copies support additional DNA repair procedures. For instance, homologous recombination (HR), which in many eukaryotes aids in haploid gamete cell variation during meiosis, also plays a role in DSB repair [81]. Surviving damaged cells may subsequently trigger carcinogenesis or additional disorders. High radiation doses and high-LET radiation such a-particles and neutrons make this process more prevalent [82].

Overall, the widely presented and aesthetically pleasing static picture of the DNA double helix obscures the reality of dynamic DNA damage and repair mechanisms, which support life on Earth and may have developed in reaction to IR, among other possible first stresses. The direct effects of background IR on DNA are probably less substantial today than they have ever been, but they may have had an impact on the evolution of life's genetic structure and DNA curation mechanisms, particularly in high-background ancient regions [83].

4.2.2 Indirect Effects

The byproducts of radiolysis, which set off a chain reaction of reactive chemicals, can also cause indirect damage to DNA from ionizing radiation. Many of these molecules are essential to life's functions; nevertheless, because of their reactivity, they may be harmful to biomolecules in addition to being valuable for signaling and defense [84]. When radiation is applied in high doses, such as during radiotherapy or the corrosion of nuclear reactor pipes, reactive oxygen species (ROS) that occur from the radiolysis of water are crucial in creating the effects [85]. The primary components of a cell, water molecules and other organic molecules are struck by radiation during indirect action, resulting in the production of free radicals such as hydroxyl and alkoxy radicals. A particularly reactive unpaired electron in the structure of free radicals makes it possible for them to react with DNA molecules and disrupt their structural integrity. Hydrogen peroxide (H_2O_2) or damages DNA molecules as well. The impairment of function or death of the cell is the outcome of radiation's indirect effect on DNA molecules. The total dosage determines the number of free radicals created by ionizing radiation. Because water makes up almost 70% of the cell, it has been discovered that the indirect action causes the bulk of radiation-induced damage [86]. In addition to the damage caused by the indirect impact, cellular damage may also be occur by reactive nitrogen species (RNS), other species and the ionization of atoms on fundamentally important molecules (Such as DNA) [87]. The development of biochemical and physiological changes that may become apparent right away or decades later is the end outcome of both direct and indirect impacts. These abnormalities may have evolved as a result of genetic and epigenetic modifications [88].

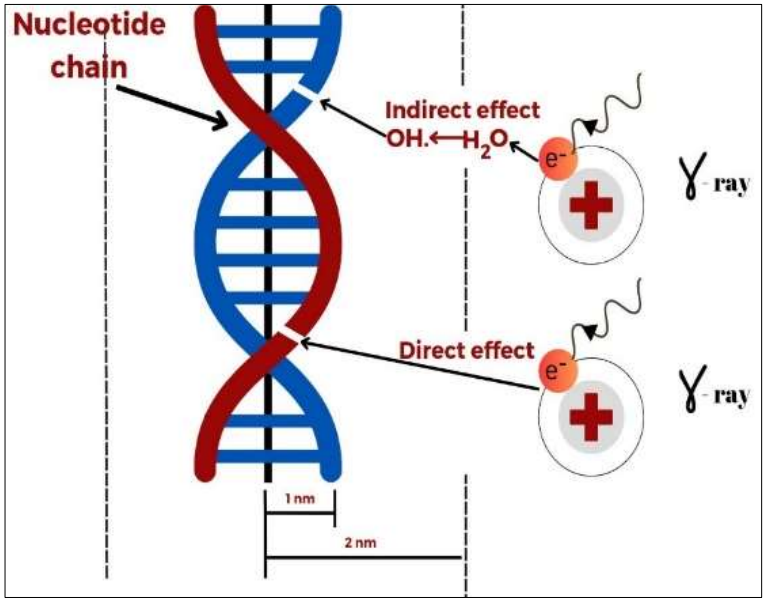

Fig 1: Direct and indirect effects of radiations (Modified) [89]

5. The Mechanism of Mutagenesis in Conventional Radiation Mutation Breeding

5.1 DNA damage caused by radiation and instability of the genome

The interaction of radiation and DNA causes direct structural and functional changes to DNA molecules through radiation energy as well as indirect damage by free radicals produced through the interactions between water molecules and ionizing radiation [90]. These interactions are the first step in the radiation mutation breeding process. Cells have developed a variety of DNA damage repair mechanisms to preserve genomic integrity. In fact, the repair strategy is used in accordance to the kind of DNA damage sustained [91]. Single-strand breaks and double-strand breaks are two different types of DNA damage. The three main SSB repair pathways are mismatch repair, base excision repair and nucleotide excision repair. DSBs, however, are primarily repaired through homologous recombination and non-homologous end joining [92]. DNA damage is different from mutation. No mutation will survive if DNA damage is correctly repaired. The process of DNA damage repair "errors," leads to gene mutations. Some of these mistakes are unintentional, such as replication errors brought by single-strand breaks that were not discovered prior to DNA replication, unstable DNA single strands during repair and involvement of low-fidelity polymerase, among other factors [90, 92]. The mutation type is essentially a point mutation with base substitution. Deletion and translocation of fragments are introduced during the repair process for severe DSBs [92]. *Figure 1* illustrates what happens if these mutations persist in subsequent cell division and are passed down to offspring. Another method of inheritance, as depicted in *Figure 1*, has the potential to produce mutant traits in offspring in addition to the direct inheritance of DNA damage brought by radiation to the progeny. The stability of the genome's genetic code is essential for preserving healthy cell division and proliferation as previously mentioned. To handle the strain on the genome brought by internal and external stress, normal cells have effective mechanisms for monitoring DNA damage and responding to keep genome damage and repair in a roughly equilibrium position. However, when this equilibrium is upset, cells enter conditions known as genomic instability, which increases their susceptibility to DNA mutations and can be brought by either genetic mutation or epigenetic modification [93, 20]. A concept known as radiation-induced genomic instability, which was first discovered *in vitro* in cell system experiments in the 1950s describes delayed and continual genetic modifications in the offspring of irradiated cells [94, 95]. Following research, it has been discovered that gamma rays, neutrons, protons and particles can cause genomic instability in cells, which is reflected by an increase in a variety of mutations, including single nucleotide mutations, an increase or decrease in the genomic copy number, gene overexpression, reconfiguration and deactivation [95, 96].

6. Determination of Effective Dosage

The main advantage of mutation breeding is the possibility of improving one or a few characters of a variety without changing the genetic background [97]. To achieve ideal results in mutagenesis, suitable mutagen doses are required. It is commonly considered that mutagen doses inducing 25-50% lethality (LD25–LD50) among M1 plants would be appropriate because they could result in the highest mutation rates [98]. However, the dose range below and above the LD50 (LD25% and LD75%) was reported [99]. Generally, LD50 and GR50 are established on the hypothesis that lower doses of gamma irradiation can produce the least impact on the plant genome which may result in morphological changes, however, higher gamma-irradiation doses may bring about several effects on the entire genome leading to negative mutations [100]. In physical mutagenesis, mutagen dose is the product of dose rate and time under constant irradiation conditions. As the biological effect of radiation appears only when the radiation energy absorbed by the organism exceeds a critical value. Nowadays, people usually use absorption dose (The energy absorbed per unit mass) rather than exposure dose (The dose of radiation applied) in plant mutagenesis [98]. It's worth noting that when we talk about a dose of chemical mutagen, we are usually referring to the amount of substance given over a set period of treatment rather, than the total quantity of mutagens in an organism or its environment. This definition has been established through studies conducted by researchers [98, 101-104]. These investigations consistently show that the rate at which organisms absorb mutagens from their surroundings depends

on factors, including the concentration of compounds in the surrounding medium and the moisture levels, within the organism cells and tissues. The same exposure dose does not necessarily mean the same dose of the received mutagen. Hence, estimation of the absorption dose is required, which would help us more precisely analyzing the biological effects of chemical mutagens, more reasonably compare results from different experiments and more properly design experiments in mutation breeding programs [105].

7. Space Mutation-Induced Mutations

Space breeding is an advanced science that combines space technology with agriculture. The utilization of unique space environment characteristics, such as particle radiation, microgravity, weak magnetism and comprehensive elements such as, high vacuum to induce advancements in agriculture biological genetics is known as space breeding. Mutagenesis is an essential radiobiological endpoint because it directly represents radiation damage to the DNA, which has the potential to affect biological diversity [106, 107]. Because cosmic radiation contains heavy ions with a wide spectrum in mass and energy, their mutagenesis potential may vary depending on the physical parameters of the ion in question. Plants cultivated in zero gravity and cosmic radiation experience physical, physiological and genetic changes, with cosmic radiation potentially being used as a technique of genetic modification [108, 109]. Since 1987, China has been actively involved in space agriculture initiatives, developing over 200 plant varieties [110]. Shiijian-8, the first space breeding satellite launched in 2006, contains 2,000 plant accessions from 133 species [111]. Cotton, maize, sunflower, cucumber, tomato, wheat, barley and soybean seed germination increased, but rice, millet, pea, sweet pepper, tobacco and lettuce exhibited no variations [112]. Through space breeding efforts, at least 66 mutant agricultural varieties have been released in China. Among them is Rice from heaven. The “Rice from heaven” was grown in the lab and the field after the seeds were returned from space and harvested.

The industrial farming sector in the United States focuses on insect and herbicide resistance, but in Asian countries, more complicated characteristics such as heat and drought tolerance and nutrient-poor soil are required. Space breeding, also known as space mutagenesis, may aid in agricultural adaptation to climate change and susceptible supply systems. Expert Liu Luxiang believes that space mutagenesis produces attractive mutations, such as the adaptive "Luyuan 502" wheat variety, which can flourish in a range of settings and conditions. Over 200 new mutant plant species, including grains, vegetables and fruits, have been approved for large-scale cultivation in China because of space breeding.

8. Significance of Mutation Breeding

The genetic diversity for desirable characteristics in different food crops is significantly increased via plant mutagenesis and mutation breeding [113-115]. One of the most effective approaches for the identification of important regulating genes and molecular pathways is induced mutagenesis. The development of novel species with enhanced agronomic traits, such as greater capacity for coping with biotic and abiotic stress and biofortification, is an essential strategy. several mutagenesis techniques have also been employed to explore the cross-link between evolution and the genetic improvement of several species, including microorganisms, animals and plants [66, 116]. In order to boost yield and quality traits which involve oil content, malting quality and the quality and size of the starch granules, a number of characters including plant height, seed dehiscence and disease resistance are essential in breeding programs. Due to the resistance to loading and high-density planting, grain output of barley, rice, wheat and maize rose. Mutation breeding has become a traditional approach in plant breeding and has contributed to the present gene pool of several crop plants and the development of new varieties with desired traits. Based on the FAO/IAEA Mutant Variety Database on the officially registered mutants, a total of 3,332 mutant crop varieties in 228 crop species have been developed [117]. The mutant varieties are improved for different traits, such as resistance to biotic stress (557), tolerance to abiotic stress (248), increased yield and yield components (1029), quality and nutrition traits (1173) and agronomic and botanic traits (2981).

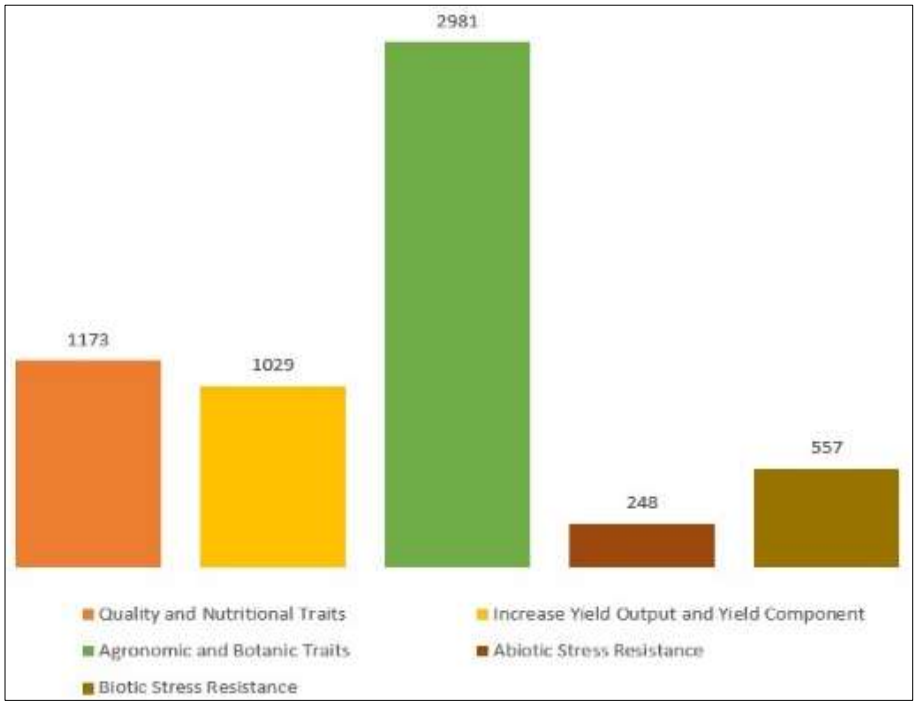

Fig 2: Registered crop mutant released by FAO/IAEA MVD, 2020

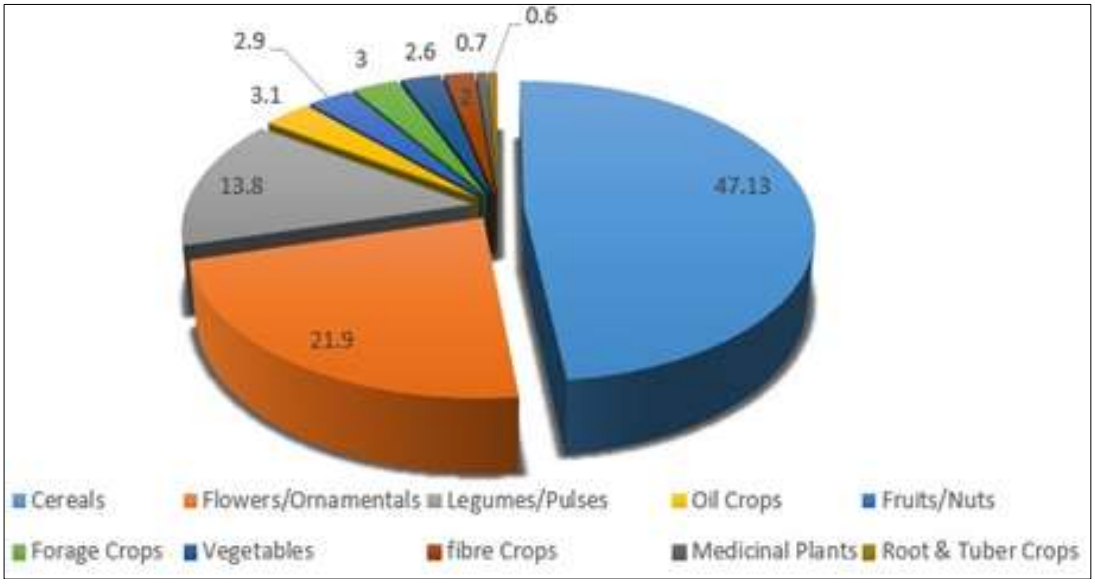

Fig 3: Different plant varieties released by FAO/IAEA MVD, July

9. Utilization of plant mutagenesis to improve crops

Induced mutagenesis is one of the most efficient tools that has been extensively utilized to create genetic variations as well as for the identification of key regulatory genes of economically important traits toward crop improvement [113, 114, 118]. It is a promising approach for the development of new varieties with improved agronomic traits, such as higher stress tolerance potential (Biotic and abiotic stress) and biofortification. Additionally, various mutagenesis approaches have been used to study the evolutionary relationship as well as for the genetic improvement of many organisms, including microbes, animals and plants [66, 116]. Any technique for mutation breeding needs to be performed in a series of steps. As shown in *Figures 2a &b*, the success of selecting desired variant mutants in the second (M2) or third (M3) generation determines the superiority of mutation breeding over other breeding techniques. To enable accurate investigation and analysis, the initial stage in mutant breeding is to significantly reduce the number of possible variants among the mutagenized seeds or other propagules of the first mutant generation (M1) [119]. Besides, the success of a mutation breeding program depends on the size of the target M1 population. A fixed targeted population size will enable a large number of measurements of mutations, therefore, breeders should efficiently manage the M1 population size. It is noteworthy that the population size is influenced by the inheritance pattern of the target gene. Therefore, to reduce the M1 population size, it is advisable to choose mutagens with a high mutation frequency [120]. Since a single mutation during treatment only affects one allele, M1 mutant plants are genetically heterozygous. However, the likelihood of obtaining a mutation on both alleles at the same time, which happens very rare, depends on the specific mutation likelihood of each allele. Furthermore, only dominant mutations in the M1 generation can be identified; at this time, it is not possible to identify a recessive mutation expression [119]. Consequently, plant breeders produce homozygous individuals expressing either dominant or recessive alleles. It is imperative to exercise prudence to avert the occurrence of cross-pollination within the M1 plants, as this can result in the emergence of novel variants that pose a challenge in differentiation from the impact of mutation [119, 121]. The process of screening and selection starts in the M2 generation and is categorized into three primary approaches, i.e., physical or mechanical, visual or phenotypical and auxiliary techniques [122]. The process of selecting seeds involves the use of physical and mechanical means to assess various characteristics such as shape, size, weight and density. On the other hand, mutant phenotypes are identified through the application of visual and phenotypical selection methods. Alternative techniques encompass physiological, biochemical, chemical and physiochemical methodologies for screening. Following the emergence of a mutant line exhibiting a favorable trait, the subsequent step entails the propagation of seeds for field evaluation. The core objective of conducting field trials is to ascertain whether the mutant exhibits the potential to emerge as a commercially viable variety that surpasses the original donor cultivar. Before being released as a commercially viable cultivar, a comprehensive analysis should be conducted on the promising mutant for its combinations of various traits, such as growth habit, structural attributes and yield components across various environmental conditions [119].

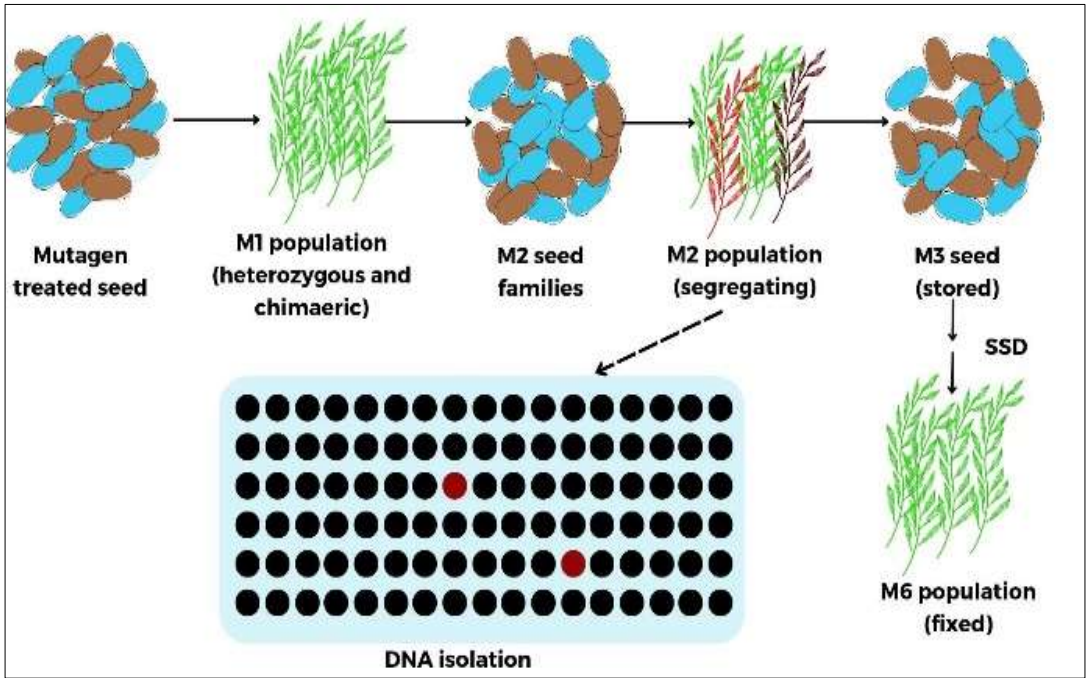

Fig 4: Authors' conceptual representation of mutagenesis by radiation

The right image depicts the mutant's formation through genomic instability, while the left image depicts the mutant's direct transmission from irradiated seeds as its source.

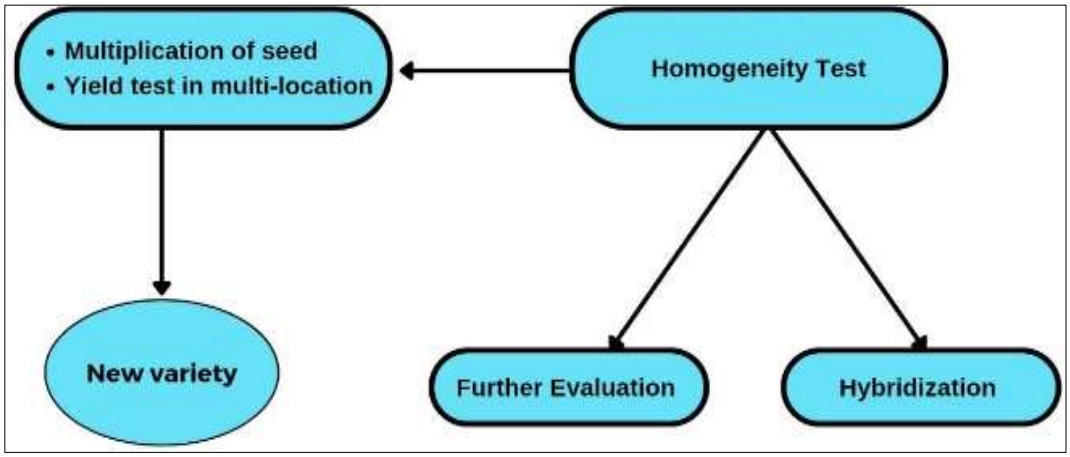

Fig 5: Genome stability test and development of a new variety.

Table 3: Induced mutation of important traits.

Crops	Improved Traits	Reference
Barley	Phytate (Ant -nutrient), salinity tolerance	[123, 124]
Canola	Improved oil quality	[124]
Maize	Resistant to obligate parasitic weed, acidity and drought tolerance; improvement of protein quality	[124]
Tomatoes	Resistant to bacterial wilt	[124]
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. Mutation of good quality	[124, 125]
Apple	Powdery Mildew and apple Scab	[126]
Sunflower	Oil quality improvement, semi-dwarf/dwarf cultivars	[124, 127]
Cotton	Resistance to bacterial blight, cotton curl virus	[128]
Rice	the dwarf and high-tillering dwarf mutants of rice	[129]
Maize	Precocious mutation, Resistance to disease and insect mutation	[130, 131]

9.1 Selection of mutagenic materials

Different organs of planting materials have been used for mutagenesis, even plant materials obtained from tissue culture [132]. However, the usefulness of these materials depends on the sensitivity of the cells to radiation [133]. Besides, the ability of the plant material to exhibit chimeras rather than complete mutated organisms.

9.1.1 Seeds

The period of quiescence, which is sometimes referred to as dormancy [134, 135], is when an organism reaches maturity and germination. This "hormesis" phenomenon, which can be driven forth by an array of environmental stresses, including ionizing radiation, is frequently seen at sub-toxic dosages. According to research, the phenomenon is widespread

regardless of the biological model, endpoint measured, inducing agent and the level of biological organization. The hypothesis that changes in the enzyme activities and an increase in the antioxidant capacity of cells are among the mechanisms of hormesis induction by irradiation is supported by several studies, even though a conclusive explanation for the stimulation effects of rays has not yet been discovered. Reactive oxygen species (ROS), which are induced by ionizing radiation and other stresses, are crucial for intracellular signal transduction and for activating the enzymatic antioxidant defense system [136]. When considering the processes that take place in a plant's life, hormones such as abscisic acid and gibberellin influence processes such as seed maturity and germination [137]. In comparison to newly air-dried seeds, aged imbibed seeds exhibit a higher frequency of induced mutations. Low water-content seeds are more radioactive. At the moment of irradiation, the seed water content must be normal. Additionally, seeds should not be too young or old either. Seed is the most often used plant portion for mutagenesis in sexually reproducible crops. Although seed germination could occur before maturity, it must first meet several conditions, including the availability of carbohydrates, proteins, lipids and nutrients, which are mostly found in the embryo [136].

9.1.2 Live plants

Due to their anchored nature and reliance on sunlight for photosynthesis, plants are naturally susceptible to solar UV radiation. In general, UV radiation induces a variety of DNA damage types that can result in mutagenesis [138]. Plants can exercise formidable strategies to react to damage [139]. For cyclobutane-type pyrimidine dimers and pyrimidine (6-4) pyrimidine photoproducts, photoreactivation, mediated by substrate-specific photolyases is thought to be the primary DNA repair pathway under light factors. Dark repair mechanisms, in contrast to photoreactivation, substitute new, undamaged nucleotides for the damaged DNA instead of immediately reversing DNA damage. Excision of dimers or their tolerance by trans lesion synthesis, both of which are replicative polymerases capable of DNA damage repair and are recognized as two alternative techniques [140]. Ultimately, frequently observed residual lesion replication lapses result in mutations [141].

9.1.3 Pollen

Among many others, mutation is one of the most formidable strategies to identify genes involved in the development of plants [142]. In microgametogensis, the correct selection of mutants for each phase is a basic step of requirement but gametophyte mutations affecting gene activities in pollen are uncommon [143]. Additionally, it is difficult to gain gametophytic mutant analysis as likened to sterile mutant, which may be due to no direct easily observable mutant phenotype and the lack of homozygous plants for mutant plant. Numerous attempts have been undertaken using various mutagenesis treatments on plants to develop techniques for inducing high frequency of mutations [144]. Frequently, seeds are used as the starter material for mutagenesis, however, pollen can help eradicate the challenge of chimerism when used instead of seeds. In higher plants, the fundamental role of the gametophytic generation is to pass on genetic material to the following sporophytic generation. The male gametophyte, which has a specific structure to carry out such a task, plays a more active and key role in the fertilization process than the female gametophyte [145-147]. Prior to physical cross-hybridization, pollen is treated with mutagenic treatment, typically in the form of irradiation, while the female partner remains somatically intact [148]. No thorough investigation of the ideal circumstances for mutant induction or the type of the resulted mutations has been done because large quantities of pollen grains (Haploid nuclei) can be easily modified and mutations are quickly passed on to the resulting generation in a hemizygous state. However, haploid pollen has special advantages for mutagenesis. A modest-sized maize tassel is thought to produce 1×107 pollen grains, while a single *Arabidopsis thaliana* flower is thought to produce 2-3×103 pollen grains [149, 150]. The M1 plants that result from pollination associated with mutated pollen are mostly nonchimeric and are hemizygous for any specially induced mutation. This can be seen as an immediate effect in the M2 progeny and because of that, fewer number of seeds are screened per plant compared to seed mutagenesis [151, 152].

9.1.4 Zygote

The plant life cycle is either a diploid or a haploid gametophyte in their generation. Flowering plants produce zygotes from fertilization which leads to the formation of the embryo. After the zygote has undergone the process of asymmetric first division [153, 154], it produces apical cells and basal cells [155, 156]. The apical cell is responsible for the formation of the embryo and the basal cell for the extra-embryonic suspensor [157, 158]. Genetic experiments in plants have brought to light the knowledge of genes that are implicated in the process of embryogenesis [159]. The first division of the zygote depends on several genes, including EMBRYONIC FACTOR 1(FAC1), which encodes an AMP deaminase and ZEUS, which encodes a thymidylate kinase [155, 160]. The partial interruption at the zygote stage was additionally observed in mutations of the DNA ligase YAO1 [161] which encodes a nucleolar protein [162], AtCDC5 [163], or Cullin 1 [164], displaying the important role of cell cycle genes in zygote divisions.

9.1.5 Scion or cuttings

The increasing domestication of food crops has been closely linked to a number of advancements in plant propagation from the dawn of agriculture. Many small-scale producers prefer on-farm grafted propagation (As opposed to buying grafted plants) because they may match specific combinations of rootstock and scion cultivars to address site-specific difficulties while supplying experts or niche markets [165]. Tomato is easy to graft compared to other vegetables [166, 167]. Competition for carbon, planting density, environmental factors and pruning control meristem development [168-172]. Through a series of intricate interactions, plant hormones control the growth of axillary meristems [173, 174]. The process of apical dominance over axillary meristems has long been linked to auxin [175, 176]. The newest class of plant hormones, stragolactones (SLs), have recently been discovered as a result of genetic mutations that exhibit greater branching [177, 178]. These mutants include the ramous (rms) mutants of pea (*Pisum sativum*), the decreased apical dominance (dad) mutants of petunia (*Petunia hybrida*), the more axillary growth (max) mutants of Arabidopsis (*Arabidopsis thaliana*) and the dwarf and high-tillering dwarf mutants of rice *(Oryza sativa*) [179-182].

9.1.6 Underground rhizomes

In vegetative cultivation, modified plant stems known as stolons and rhizomes serve this purpose. As stolons grow

above the soil, rhizomes grow underground into the soil [183]. These plant parts have genotypes that make it important for propagation and material for mutagenesis. The meristem that results from the rhizome has a node or at the developmental phase, the rhizomes bend to produce a vegetative clone at the tip [184]. Rhizomatousness and a perennial life strategy go hand in hand. Rhizomes store and distribute nutrients for a constant development and shield underground-dormant buds from predators throughout the winter. It is interesting to note that rhizomatous traits are lacking in grain-producing annual plants such as sorghum, rice and maize, although each of these plants has a closely related, perennial and rhizomatous relative, *Sorghum propinquum*, *Oryza longistaminata* and *Zea diploperennis*. The majority of rhizome-forming quantitative traits loci (QTLs) in sorghum and rice show strong correlations, indicating that some of the same genes may influence rhizomatous traits in these distantly related grass species. This discovery supports the theory that cultivated sorghum and rice, which have an annual habit, may have developed from their perennial, rhizomatous ancestors through gene mutations that are similar to those that caused the rhizomatous traits [185, 186].

9.1.7 *In vitro* tissue

The rate of the accumulation of spontaneous mutations is accelerated by the unique setting of *in vitro* culture settings, tissue reprograming and the disorganized development that is usually associated with *in vitro* culture, especially when callus formation is involved [187]. Random *in vitro* mutagenesis techniques offer advantages such as uniformity, easy application of selective agents, small space and disease-free plant material handling [188]. When employing *in vitro* mutagenesis techniques, soma-clonal variations can be used with random mutagenesis methods to further boost the mutation frequency. Random mutations carried out *in vitro* on plant cell cultures makes it easier to select certain agronomic traits, such as resistance to herbicides, salts, metal, flooding, cold and drought, or the selection of the embryo-gametophyte-lethal mutations for asexually reproducible plants [189]. The development of chimeras after the mutagenic treatment of multicellular organisms is one of the main problems of mutation breeding in higher plants. Therefore, mutant selection techniques using cell culture are more effective [126, 190]. By extracting chimeric cells produced by induced mutagenesis in chrysanthemums, a method based on *in vitro* culture was used to isolate unusual ornamental forms. Other ornamentals can also benefit from this technology [191]. *In vitro*-selected lines are referred to as variations and they can be used to examine the source of phenotypic alteration (Mutation or epigenetic change) [190, 192].

10. High and low energy particle mutation breeding

The history of high-energy particle mutation breeding spans almost 30 years. The phenotype of ornamental plants including sterility, flower color and shape was improved using the earliest high-energy particle radiation mutagenesis techniques. New flower cultivars, such as the verbena sterile cultivar and the chrysanthemum, dahlia and rose color or shape cultivars, have been generated since 2002 [193]. The development of agricultural products with exceptional traits, such as dwarfed buckwheat, barley, pepper [193], tearless and pungent onions [194], lettuce with low browning traits [195] and rice with a stay-green phenotype [196], has also made extensive use of high-energy particles. Finding the best physical radiation parameters, such as radiation dose and LET, which are crucial factors to be considered in particle radiation mutagenesis, is necessary to increase the efficiency of this technology. The rates of survival of model plants and microbes decline with dose, so the physical radiation parameters best suited for mutagenesis must strike a balance between survival rate and mutation frequency. For instance, using the model plant *Arabidopsis thaliana*, it was demonstrated that the maximum number of mutants can be produced using a 30 keV/m LET carbon ion beam and a 300-400 Gy irradiation dose [197]. A smaller LET is better in inducing small deletions, but larger LET radiation would result in large deletions, according to additional mechanistic studies at the genomic level in model plants and model microbes [198, 199]. Furthermore, the relatively high LET Ar ions can result in more complex rearrangement errors in *Arabidopsis thaliana* than C-ion irradiation technology through whole genome sequencing [197]. A large number of SSBs and DSBs are the main types of DNA damage caused by high-energy particle radiation. In general, mutations caused by damage are usually incorrectly repaired. While DSBs represent damage that has the greatest impact on DNA and typically requires more time to repair, SSBs can be quickly and easily fixed [200, 201].

Radiobiology has always placed a high priority on the biological effects of particle irradiation. However, because of their extremely shallow depth of penetration in matter, low-energy particles (10–200 keV) have long been underappreciated. This leads to the theory that high-level biological effects cannot be induced through their interaction with organisms. The genetic impact of low-energy particle implantation on rice was first verified by Yu *et al*. in the early 1980s [202, 203]. The question of how low-energy particle implantation causes mutations still needs to be answered. Low-energy particles have been used in breeding for years and it has been discovered that they are a highly effective source of genetic modification by mutagenesis, leading to notable successes [193, 204] and the emergence of a new interdisciplinary field called low-energy particle biology [205]. The four-factor theory of energy absorption, mass deposition, momentum transfer and charge neutralization, which Yu *et al*. proposed in the 1990s, states that energetic ions are transferred into organisms and cause serious etching to cells and physical damage to biological macromolecules [205]. Ion channel and soft X-ray theory were combined to provide an explanation of the physical interaction process. Further explanation of the role of the biological process in the mutagenesis caused by low-energy particle implantation followed. The radiation-induced bystander effect (RIBE), which has a theoretical range of 1 m for low-energy particles in water but could hardly penetrate the seed coat, may be responsible for any potential biological genetic effects. When nearby irradiated cells send signals to nearby nonirradiated cells, the nonirradiated cells respond biologically [206]. This phenomenon is known as RIBEs. To test this theory, only the middle of each Arabidopsis seed was exposed to radiation while the shoot apical meristem (SAM) and root apical meristem (RAM) were shielded. Several postembryonic development endpoints of SAM and RAM were inhibited following 30 KeV 40Ar irradiation. In a different investigation, various Arabidopsis R3L66 seedlings (SAM-, RAM-, cotyledon- and radicle-oriented) were exposed to radiation. Significant increases in genetic changes were found in the irradiated plants' nonirradiated aerial parts [207]. These findings demonstrated that plants could experience long-distance bystander effects. These mechanistic studies have

provided strong support for defining the biological effects caused by low-energy particle irradiation by illuminating the temporal and spatial characteristics as well as the molecular mechanism of radiation bystander signals in plants [207, 208]. The trait variation induced by low-energy particles is currently linked to radiation parameters using big data analysis technology. The randomness of mutation is anticipated to be overcome and further advancement in the field of low-energy particle mutation breeding is encouraged by modifying radiation parameters, such as the type of irradiated particles, dose and energy.

11. Identification of Radiation Mutants

11.1 Morphological identification

To create mutant plants with desirable plant features, induced mutagenesis is performed on a regular basis [209]. Mutant cultivars with improvements from several crop species have been frequently used in agriculture [210, 211]. Induced mutagenesis is frequently employed in plant breeding and has emerged as a key tool for the creation of better cultivars. Depending on the dose, different mutagens have been reported to have diverse effects on a plant's physiology, anatomy, biochemistry and morphology. Studies have employed mutagens to improve morphological variations, including soybean herbicide resistance, spring rape early flowering, wheat male sterility and tomato smaller fruit size [212-214]. It is believed that morphological traits are an effective technique to distinguish between mutant plants and wild species. Because morphological mutants are crucial for modifying cultivar characteristics and generating new types of plants, the morphological qualities of plants have been utilized to detect alterations in a variety of plants. Mutagens give the chance to increase the genetic diversity of quantitatively inherited traits, which has been suggested as a feasible solution to problems with plant cultivation [79].

In all hybridization projects, the selection of desired traits involves the use of morphological mutants. Each gene of agronomic importance can be mutated, according to research; as a result, mutation tests should produce a wide range of viable mutants that are morphological in nature [31]. According to the segregation pattern of morphological mutants, the majority of the true breeding mutants were influenced by a single recessive gene, however, other research argued that the various morphological mutants that bred true in subsequent generations, such as tall, dwarf, semi-dwarf, bushy, prostate and bold seeded mutant types, were found to be under the influence of polygenes. Increases in mutagen dose have been demonstrated to enhance the frequency of morphological mutations [215]. According to research, the range of feasible mutations was greater at lower mutagen dosages [216]. The study found that EMS treatments and medium doses of gamma rays resulted in a higher frequency of viable mutations [217, 218]. The range and magnitude of changes, in an organism's structure, known as "mutations" are affected by factors when they are exposed to different mutagens for varying periods of time and in diverse genetic backgrounds of the subjects used in experiments. This information is crucial, for understanding how structural alterations can occur and their severity after being exposed to these agents [218, 219].

11.2 Physiological and biochemistry identification

The modification of physiological features can benefit from irradiation [220]. The interaction of gamma rays with atoms or molecules in the cell, notably water, results in the production of free radicals, which have a biological effect [221]. Depending on the radiation dose, these radicals have been reported to have a variety of impacts on the plant system, including the ability to harm or change vital plant cell components [222]. These outcomes include modifications to the plant's cellular structure and metabolism, such as thylakoid membrane dilatation, altered photosynthesis, oxidative stress modulation and accumulation of phenolic chemicals [221, 223, 224]. Irradiation by gamma has photons that are potent enough to interact with any type of molecular entity without discrimination. According to earlier studies, metabolic activity and hydrolyzing enzyme activity in germinated seed which lead to a decrease in the total protein and carbohydrate contents were increased with increasing irradiation dosage. [225, 226]. Except for serine and valine, which increased at 100 krad (1 Gy), radiation decreases the anabolic utilization of all substrates and the levels of all amino acids [34]. The velocity of pectin and alginate could be decreased by irradiation at dosages of 15 to 30 kGy [227]. The dry seeds of Bengal gram (*Cicer arietinum* L.), horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) and cowpea (*Vigna unguiculata* (L.) showed slight differences in the breakdown of oligosaccharides in processed legumes when compared to the control [228]. The seed protein is broken down and more amino acids are produced by gamma irradiation [225, 220, 226]. The synthesis of proteins may be inhibited by this process. In wheat and rice plants, total proteins and carbohydrates fell as high gamma rays dosage was increased [34, 229]. The retention of proteins in the plasma membrane of muskmelon (*Cucumis melo* L.) fruit 10 days after exposure to 1 kGy irradiation was the subject of extensive research [230]. A study was performed on how g-irradiation affected the levels of total free amino acid nitrogen in five different kinds of Iraqi dates (*Phoenix dactylifera* L.). Recent research investigations conducted have shown that certain amino acids, including proline, glutamic acid, aspartic acid, serine, histidine, lysine and tyrosine are particularly sensitive, to changes when exposed to ionizing radiation [231, 232]. On the hand methionine, isoleucine and leucine displayed a slight increase in radiosensitivity. Interestingly studies by [233, 234] have revealed that plant cells have a defense mechanism triggered by ionizing radiation exposure which could contribute to the observed changes, in amino acid composition. The way this process works is by speeding up the production of substances or enzymes that contain sulfur, such as superoxide dismutase, glycation and amino acids, which can defend against free radicals, neutralize them and participate in the simultaneous release of protective substances in an organism [235].

By enhancing the activating enzyme system, low dosages of radiation such as gamma rays increases the production of chlorophyll. The improvement of yield components and chlorophyll parameters in different plants, including tomato (*Lycopersicon esculentum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), was induced after variable doses of g-rays, according to these results, which were almost in agreement with those of other groups [236-239]. Higher gamma irradiation prevents wheat from synthesizing chlorophyll and older leaves of *Holcus lanatus* L. exposed to 40, 80 and 160 Gy showed alterations in coloration [240, 241]. Etiolated barley and wheat leaves in potato tubers served as a demonstration of this. It was discovered that Gamm irradiation (1 kGy) affected the chloroplast structure characteristically in fruits (*Hardenpont pears*) that had chloroplasts in the hypodermis at the time of harvest [34]. The quantity of chlorophyll in the oil processing was reduced

or eliminated using irradiation technology without creating lipid peroxidation during irradiation [242].

11.3 Cytological identification

Certain chromosomal proteins are directly altered by some mutagens and these chromosomal aberrations take place during meiotic division [243]. Following irradiation of *T. aestivum*, chromosomal aberrations, including laggards, c-mitosis, multipolar chromosomes with or without spindles, stickiness, premature bivalent, tripolar cells, fragments and bridges, disjunction and micronuclei appeared [244-246]. There was significant chromosomal stickiness during metaphase I, and an increase in the radiation dose resulted in a significant proportionate increase of all abnormal cells with chromosomal stickiness [247, 248]. Additionally, plants exposed to gamma radiation showed other abnormalities such as unipolarity at metaphase I. Micronuclei started to appear as a result of the abnormalities that were seen in the earlier stages of the meiotic cycle. The abnormality may be caused by fragments or laggards, which could change the number and size of pollen grains produced from the microspore mother cells. The presence of micronuclei can indicate how severe genetic changes caused by radiation are, in plant cells. There is a connection between radiation exposure and the occurrence of micronuclei meaning that higher levels of radiation are linked to instances of these abnormal nuclei. This suggests that exposure to radiation has effects on the DNA of plant cells resulting in abnormalities or mutations. It's worth noting that studies have demonstrated counts of micronuclei at relatively low doses of radiation which implies that radiation can cause significant genetic harm to plants, at certain levels [248]. The clustering of any cell cycle phase is a defining feature of chromosome stickiness. Numerous pyknotic nuclei could form in the case of extreme stickiness. Genetic or environmental factors may contribute to chromosome stickiness [249]. According to some researchers, the chromatin fibers fail to properly condense during the synthesis stage, which leaves them open to being caught and entangled with fibers from other chromosomes and forming a physical connection. The stickiness is the result of many chromosomes adhering to one another as a result of such abnormal chromatid connections [248, 250]. According to research, cells with laggard chromosomes may occur as a result of abnormal spindle formation where the chromosomes are not transported by the spindle fibers to the polar region [251]. The existence of fragments may be explained by the broken chromosome's inability to be recombined [252].

12. Summary and Prospects

In light of the mounting demand for nutritious food and high-yielding crop varieties amidst shifting climatic conditions, plant breeding research is gaining prominence through integrating biotechnology and molecular genetics. To address the challenge of creating resilient crops, mutation breeding approaches have gained popularity as an alternative to conventional breeding techniques. This involves combining desirable traits from multiple sources via *in vitro* mutagenesis, which has become a valuable tool for improving crop quality. Advanced genomic techniques enabled by next-generation sequencing enable plant breeders to explore novel genetic pathways and uncover previously unknown genes. These insights will contribute significantly to enhancing crop improvement strategies aimed at amplifying heritable variation. While other mutation breeding methods face developmental bottlenecks, their applications in agriculture have already led to substantial gains in crop yield and elucidated key regulatory processes. Nonetheless, accelerated progress in mutation breeding research depends critically upon integration with emerging technologies such as CRISPR/Cas9 gene editing. Moreover, given the projected rise in global populations, declining arable landmass, and environmental degradation, stress-tolerant crop cultivars remain indispensable for ensuring long-term food security.

To further shorten the crop breeding cycle and increase breeding efficiency, we should emphasize the mutual development and joint application of multiple breeding approaches. Since many breeding traits are complex quantitative traits, gene editing or molecular breeding methods based on a small number of genes are not the best options for improving quantitative traits. As a result, mutation breeding research must be expanded. Radiation mutation breeding does have some drawbacks, including the beneficial mutant frequency being relatively low and the difficulty in predicting the direction and nature of variation. Indeed, there are still scientific issues that require attention, such as creating more beneficial varieties and understanding radiation mutagenesis mechanisms.

In-depth research is still needed, including the causes of mutations in progeny plants, because our knowledge of the radiation breeding mutagenesis mechanism is still insufficient. Genomic instability, which cause mutations as already mentioned, is a double-edged sword for crop breeding because it can both increase the rate of variations in the offspring and cause the instability of mutant traits. Therefore, it is important to understand how genomic instability affects plant mutagenesis. It is anticipated that, with the development of advanced radiation devices, high throughput gene sequencing and other forms of advanced molecular biotechnology, the mutagenic effects of radiation may one day be predictable, allowing research to progress toward directional mutagenesis. A key prerequisite for the implementation of modern radiation mutation breeding is the establishment of an accelerated particle radiation device and its varied parameters.

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