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Mutation breeding in the ERA of genomics: New frontiers in horticultural research

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Abstract
Mutations can arise from changes in DNA or from the process of cell division or replication. When it is desirable to improve one or two traits of a variety with good adaptability, the use of mutations in direct plant breeding becomes more significant. The significance of mutant breeding in horticulture crops is addressed in this paper. It assesses novel biotechnology strategies and potential mutation breeding. A new era of crop enhancement mutation breeding is being ushered in by molecular mutation breeding. Mutation breeding will be essential in improving crops and addressing issues with global food security in the upcoming years and decades. Therefore, the purpose of this review study is to assess the role of mutant breeding in horticulture research and examine its potential applications.

Keywords: Mutation, genomics, horticultural crops, research, breeding

1. Introduction
"Mutation" is one of the approaches to produce variation in genetics. Mutations are any alterations to the genetic structure. According to Salgotra and Chauhan (2023) [43], mutations are hereditary alterations in the DNA sequence that arise from modifications in the physical and chemical structure of the hereditary material rather than via genetic expansion or recombination. A mutation is any alteration to an organism's DNA that is not brought about by regular recombination and segregation. Possible causes include errors generated during normal cell division and replication, as well as exposure to mutagenic agents like radiation or certain chemicals. Plant evolution is based on natural selection, hybridization, and mutation.

Plant breeding has a long history dating back to the early days of agriculture. Humankind began breeding plants thousands of years ago. People learnt to plant seeds in the soil at specific times of the year during the pre-agricultural era. This resulted in the initial plant production and the start of plant agriculture. Natural variety from various genetic resources and traditional breeding techniques (selection, hybridisation, etc.) has been used to create cultivated plants (Cooper *et al.* 2014; Machado *et al.*, 2011) [9, 27]. Spontaneous (naturally occurring) mutations were used to generate the first breeding successes. The most well-known semi-dwarf rice and wheat mutants were used during the 'Green Revolution.' In plant molecular biology, induced mutagenesis is gaining popularity as a technique for locating and isolating genes as well as researching their makeup and functionality.

There are numerous genetic methods that can be used in place of the natural transfer of genes, including genetic engineering and selection. Among the accomplishments of transgenic technology are the creation of pesticide-resistant goods, the acquisition of genes that provide resistance against viral infections with the assistance of virus coat proteins, and the acceleration of the nutrient level of farmed plants (Rauf *et al.*, 2010; Ozudogru *et al.*, 2011; Stuthman, 2002) [35, 40, 48].

Creating variation in desired genotypes, selecting through selection, observing, and multiplying are all part of contemporary breeding research. To improve productivity and reduce the amount of time needed for breeding, plant breeders combine a number of strategies. Different plants can be produced by the crossing process between the parents in the population, or "Genetic Variation" can be produced by causing mutations in the individuals who make up the population (Schleif *et al.*, 2021; Salgotra and Chauhan, 2023) [45, 43].

Numerous advantageous qualities are present in horticultural crops for human consumption. Horticultural crops are now the focus of research in an effort to increase resilience and sustainability in addition to productivity and quality (Lastochkina *et al.*, 2022) [22]. Many horticultural plant species are currently the subject of breeding experiments using both conventional and modern biotechnological methods.

2. Mutation Breeding

According to Van Harten (1998) [50], mutations are abrupt heritable changes in an organism's genetic material and ensuing phenotypes that are different from genetic segregation or recombination. Mutations that arise spontaneously are uncommon and happen at random in terms of the genes they affect and the time they occur. The use of radiation-induced mutations to improve fruit crops and increase their resilience in the face of climate change is being investigated (Maan *et al.*, 2023) [26]. These mutations have detrimental effects that make the organism less adapted to its environment and, in the worst situations, can be fatal.

A scholar named Hugo de Vries initially proposed the concept of using these mutant types for one's own advantage in his 1901 work, Mutation Theory, which described how mutation creates new types in plants and animals. Plant breeders began using induced mutations to alter plant traits in the next years (Guldag *et al.*, 2023; Ahloowalia *et al.*, 2004; Micke *et al.*, 1987) [14, 1, 30].

Finding high-yield and high-quality varieties that are suited to the soil and climate of wide regions, or enhancing the shortcomings of the current types, is the goal of breeders when they introduce new varieties. Breeders use their newly created tactics and procedures as well as natural variations to their advantage. Many new useful types have been introduced to the agricultural industry through the use of conventional breeding techniques, which represent one of these new approaches. It frequently takes a lot of time, money, and effort to produce variations using these traditional breeding techniques. In order to save time, create a planned study, and quickly produce new types, the mutant breeding method has begun to be applied (Wang *et al.*, 2019; Jonas *et al.*, 2016; Nerkar *et al.*, 2022) [51, 17, 31].

Mutation breeding's broad influence on agricultural highlights, its versatility and usefulness, especially when it comes to fruit crops. Breeders can successfully diversify plant characteristics by inducing mutations, which leads to improved varietal traits, amplified yield, increased resistance to diseases, and increased resilience to biological and environmental stresses (Singha and Singha, 2024) [46].

Induced mutant breeding has gained attention because of the prevalence of spontaneous bud mutations, also referred to as bud sports, in fruit crops, especially in citrus, mango, and grapes. Variety-introducing mutations, which may lead to chromosomal deletions, inversions, translocations, and nucleotide base changes, propel the evolution of new forms, varieties, or species. Physical and chemical mutagens including gamma rays, x-rays, and EMS (ethyl methane sulphonate) can cause mutations artificially. Because of its heterozygosity, long juvenile stage, and perennial nature, conventional breeding has limitations in producing fruit harvests. Overcoming these challenges and boosting genetic variety can be accomplished through induced mutation breeding. Prolonged heterozygosity, copious fruit, incompatibility, drop, polyploidy, apomixis, and the juvenile period are some of the challenges faced by traditional methods.

In order to advance fruit tree genetics, genetic variety—whether natural or artificial—is essential. Mutational breeding works well for creating horticulture variations by employing chemical and physical mutagens to add desired traits. Changes in blooming time, fruit ripening, colour changes, dwarfism induction, self-compatibility, seedlessness, self-thinning, and disease resistance are examples of successful outcomes. Benefits of mutation breeding include improved variety, ploidy level induction in fertility restoration and adapted species, sterile hybrids, improved flavour and fragrance, and larger fruit.

Mutation breeding has been revitalised by the quick development of plant molecular genetics and genomics, which has made it a key crop improvement tactic. According to predictions, continued advancements in these areas will immediately help mutant breeding by enabling breeders to fully utilise genetic diversity for trait development (Jegadeesan and Punniyamoorthy, 2023) [16]. However, the availability of a variety of genetic reservoirs suited to particular features is a crucial requirement for the effectiveness of plant breeding. All things considered, the combination of genetic engineering, molecular biology, and mutant breeding heralds a new age in crop development by giving breeders hitherto unheard-of resources to address issues related to global food security and support agricultural sustainability (Wenefrida *et al*., 2013) [53].

3. Mutation Techniques in Horticultural Plants

A successful mutation breeding program aims to develop a recognisable morphological marker for promising or even cultivar registration, improve the cultivar or even one or a few characters, restore male sterility or fertility for the creation of useful hybrid varieties, and produce mutations that are easy to inherit (Hao *et al.*, 2021; Kaya *et al*., 2018) [55, 19].

Mutation breeding studies depend on the availability of the mutagen, the amount of material to be applied, and the mutagen's capacity to induce mutations. A mutagen is a physical or chemical substance that can alter an organism's DNA permanently by causing mutations. To obtain genetic diversity for mutant breeding experiments, a variety of physical and chemical mutagens are employed. X-rays, gamma-rays, neutrons, beta cathode rays, alpha particles, and protons are examples of physical mutagens. There are two types of these mutagens: slow ionising and quick ionising. UV light, X-rays, and gamma rays from radioactive isotopes like cobalt-60 or cesium-137 are examples of slowly ionising substances. B particles and basic or slow neutrons are examples of fast ionisers. The genetic makeup of plant material is drastically altered by these mutagens. The plant's chances of surviving are decreased by these alterations, which result in chromosome breakage (Micke *et al*., 1987; Ahloowalia and Maluszynski, 2001) [30, 1].

Ane (2014) [3] also studied the effect of gamma radiation on yield due to yield in two varieties of peas (*Pisum sativum* L.). Dose-dependent reduction in yield is observed in most of the two cultivars, but the genotype of var. *arvense* is known to be more sensitive to gamma radiation doses than var. *hortense.* Physical mutagenesis are used more often than chemical mutagens and in physical mutagens, γ-rays are more commonly used than X-rays (Beyaz and Yildiz 2017) [6]. Mutation screening is the selection of individuals from a large mutational population that meet specific selection criteria, and mutation confirmation is being re-evaluated (Oladosu *et al.* 2016) [33].

Table 1: Physical Mutagens Used for Inducing Mutation

Fruits	Mutagen	Sources	Plant Material	Lethal Dosages	References
Banana	Gamma Rays	Radioisotopes and Nuclear Reaction	Shoot Tip	30-35 (Triploid)	Lamo *et al.*, (2017) [21]
Japanese Plum	Gamma Rays	Radioisotopes and Nuclear Reaction	Shoots	30	Predieri and Gatti (2000) [38]; Lamo *et al.*, (2017) [21]
Pear	X-Rays	X-Rays Machine	Shoot Tip	11	Rui Liu (2022) [42]; Maurya *et al.*,(2022) [28]
Apricot	Thermal Neutrons	Nuclear Reactor	Pollen	>75-<100	Wu *et al.*, (2011) [54]; Maurya *et al.*, (2022) [28]
Apple	Gamma Rays	Radioisotopes and Nuclear Reaction	Leaves	>10-<20	UluKapi, & Nasircilar, (2015) [49] Lamo *et al.*, (2017) [21]
Cherry	X-Rays	X-Rays Machine	Shoots	>94-<98	Maurya *et al.*,(2022) [28];
Strawberry	Gamma Rays	Radioisotopes and Nuclear Reaction	Shoots Clumps	>50-<100	Lamo *et al.*, (2017) [21]
Kiwi	Gamma Rays	Radioisotopes and Nuclear Reaction	Leaves	40-60	Pathirana, R. (2021) [36].

According to Mba *et al.* (2010) [29], chemical mutagens are generally thought to have a milder impact on plant materials. These agents have the benefit of being easy to apply because they don't need complex facilities or equipment. Additionally, compared to physical mutagens, chemical mutagens typically have a greater ratio of desired mutations to undesired modifications (Oladosu *et al.*, 2016) [33]. However, because chromosome abnormalities and the related negative effects of ionising radiation were becoming more common, researchers started looking for other ways to produce mutations. As a result, numerous chemical mutagens have been discovered. Diethyl sulphate, ethyl methane sulphate, methyl methanesulfate, ethylenimemine, N-nitrose N-ethylurea, and azides are examples of chemical mutagens. Since chemical mutagens can produce micromutations, they are typically used (Luch, 2005; Kodym and Afza, 2003) [25, 20].

Although these mutagens have a number of industrial and research uses, it is important to carefully handle any potential hazards or adverse effects. One of the most popular chemical mutagens for causing mutations in plants, such as grapes and bananas, is ethyl methane sulphonate (EMS), an alkylating agent (Gado *et al.*, 2018) [12]. The effectiveness and efficiency of EMS in creating somaclonal variations in agricultural plants are well established. Banana shoot tips are regularly exposed to mutagens during the process, which leads to their regeneration (Penna *et al.*, 2019; Rajan and Singh, 2021) [37, 39]. In bananas (*Musa* spp. AAA group), chemical mutagens have also been used to create fusarium wilt-resistant cultivars (Saraswathi *et al.*, 2016) [44].

Table 2: Chemicals used for Inducting Mutation

Mutagen group	Mode of Action	Example
Alkylating	By adding methyl or ethyl groups to bases, the alkylated base can undergo degradation, producing a basic site that is both mutagenic and recombinogenic. Alternatively, it may mispair during DNA replication, leading to mutations, with the outcome dependent on the specific atom affected.	1-methyl-1-nitrosourea (MNU); 1-ethyl-1-nitrosourea (ENU); methyl methane sulphonates (MMS); ethyl methane sulphonates (EMS); dimethyl sulphate (DMS); diethyl sulphate (DES); 1-methyl-2-nitro-1-nitrosoguanidine (MNNG); 1-ethyl-2-nitro-1-nitrosoguanidine (ENNG); N,N-dimethyl nitrous amide (NDMA); N,N-diethyl nitrous amide (NDEA).
Azide	Similar as alkylating agents	Sodium azide
Hydroxylamine	Similar as alkylating agents	Hydroxylamine
Antibiotic	Chromosomal abnormalities have also been associated with male sterility.	Actinomycin D; streptonigrin; mitomycin C; azaserine
Nitrous Acid	Deamination occurs when cytosine is replaced by uracil, which can combine with adenine and lead to transitions in following replication cycles.	Nitrous acid
Acridines	They insert themselves between DNA bases, disrupting the DNA double helix structure. DNA polymerase perceives this distortion as an additional base and inserts another base opposite the intercalated molecule. This process leads to frame shifts, altering the reading frame of the DNA sequence.	Acridines orange
Base Analogues	During the process of DNA replication, there is the substitution of the regular bases with others, resulting in transitions (such as purine to purine or pyrimidine to pyrimidine), as well as tautomerization, where bases exist in two interchangeable forms (for example, guanine can be present in either keto or enol forms).	5-bromodeoxyuridine; 5-bromouracil (5-BU); maleic hydrazide; 2-aminopurine (2AP)

Transposable elements are an additional mutation tool. A vast variety of DNA sequences are found in transposable genetic elements, and each one has the ability to move to other areas of genomes either directly (transposons) or indirectly (retrotransposons) through an RNA intermediary. When biologist Barbara McClintock first identified it in maize plants in the middle of the 1940s, it was thought to be a genetic abnormality. The anthropomorphic terms "selfish" and "parasitic" were applied to TEs a few decades later due to their capacity for genetic disruption and replicative autonomy. Important transposable mutagens include T-DNA, retroviruses, transposons, and retrotransposons (Ahloowalia and Maluszynski, 2001; Brock, 1997) [1, 7].

3.1 Selection of Breeding Variety

Mutation breeding, mixing, and lowering foreign pollination should all be done at the same time when choosing the primary variety. Additionally, it could be a recently registered variety, a promising line that has to be written, an introductory variety, or an excellent line that was taken off the registration list because it lacked certain qualities. Cracking, colour changes, winter and summer traits including early or late maturity, and short and tall plant traits are some of these characteristics (Kaya *et al*., 2018; Jung and Till, 2021) [19, 18].

3.2 Planning the M_1 Generation

Determining the dose limit to be employed in greenhouse, lab, and field trials should be the initial step in mutant breeding research. It must be cultivated in both the control and mutagen-treated populations during field tests. It eliminates phenotypic changes in the parent variety and permits comparison of treatment effects on control populations, germination, growth, survival, damage, and sterility in M1 (Noman *et al.*, 2017) [32].

It is advised to utilize two distinct mutagens and at least three dosages when applying mutagens, excluding control. The dose limitations that can be utilised in mutant breeding are 20% more or 20% less than the 50% growth-reducing dose found under greenhouse and laboratory conditions. Amounts that reduce the growth of chemical mutagens by 10-30% and ionising radiation by 15-30% are used. The M1 population should be sufficiently large to produce mutations at the anticipated rate (Zhao *et al*., 2023) [55].

Careful consideration should be given to field preparation, planting time, ambient temperature, and weed management before sowing seeds in the M1 generation. To ascertain the impact of mutagens on the M1 generation, prompt observations are required. The goal should guide the selection process, beginning with the M2 generation. It is appropriate to use the pedigree approach on M2, M3, and M4 generations. Once they have been proved to settle, those that retain their heredity and exhibit superiority in terms of desired traits are either employed as rootstocks in hybridization or taken into yield trials (Noman *et al.*, 2017) [32].

4. A Current Approach in Plant Breeding: CRISPR/Cas9

Some plant species have employed the CRISPR/Cas9 gene editing technique for a variety of purposes, most notably yield enhancement and the control of biotic and abiotic stress. Since they explain how the CRISPR/Cas9 system is used to disable particular genes that are crucial for tolerance mechanisms to biotic or abiotic stress, the majority of these studies are regarded as proof-of-concept studies. The development of disease-resistant plants is severely hampered by the biotic stress caused by pathogenic microbes, which also account for about half of the potential yield loss and contribute to worldwide decreases in food production. Genome editing using CRISPR/Cas9 has improved tolerance to significant abiotic conditions like drought and salinity and raised resilience to plant diseases (Wang, 2020; Ricroch *et al*., 2017; Li *et al*., 2022) [52, 23, 41].

Compared to traditional breeding, scientists can now add desirable features more rapidly and precisely thanks to new breeding procedures. Plant breeding and research have been revolutionised by genome editing technologies based on CRISPR/Cas9. It has recently been used to enhance important agricultural characteristics, like functional research and the defence against biotic and abiotic challenges in a variety of plant species. However, the CRISPR/Cas9 system's potential has not yet been completely investigated, despite the fact that the CRISPR/Cas9 vector platform has been built and its effectiveness in genome editing has been tested. The majority of the work done is preliminary and requires refinement, even though several changes to this technology have resulted in targeted productivity gains. CRISPR/Cas9 genome editing technology will be a crucial area of research to use in plant breeding in the future to improve agronomic qualities such as disease resistance, yield, and nutritional value (Ahmad *et al*., 2020; Çiçek *et al*., 2023) [2, 8].

5. Artificial Intelligence Technologies in Plant Breeding

Artificial intelligence involves the use of man-made instruments, such as computers and robots, to mimic natural systems, such as humans and animals. This approach entails comprehending the representation of information in order to store it in computer memory and enable automatic deductions from it. It also entails knowing how choices may be made, how plans of action can be developed using information that has been saved, and how computerisable information can be gathered by analysing sample data or consulting with human specialists. Artificial intelligence systems are machines that have been programmed to possess human intelligence skills like perception, learning, information gathering, reasoning, and decision-making. Using computer models, they analyze and develop human intelligence-related mental processes and apply them to other systems (Dong *et al.*, 2021; Benos *et al.*, 2021; Bačiulienė *et al.*, 2023) [11, 4, 5,].

The main objective of agricultural production is to manage plant and animal production in a way that is profitable, sustainable, and productive. In order to achieve this, alternative solutions are created to facilitate agricultural operations and issues that need to be resolved or improved through the use of technology in a variety of areas, including boosting agricultural productivity and product quality, utilising the fewest possible inputs, ensuring food safety, and safeguarding the environment and natural resources. Smart machines and production systems that control devices have started to replace conventional production methods throughout the agricultural production development period because of the quick advancement of information technologies following mechanisation, automation, control, and informatics. In order to control the processes of gathering, processing, storing, transporting, and using information, information technologies are made up of hardware, algorithms, and software. Applications that demand precision, like breeding, have been made simple by combining the current understanding and expertise in agriculture with the machine learning, deep learning, artificial intelligence, modelling, and simulation tools provided by information technologies. Applications of artificial intelligence are anticipated to be used in the most significant agricultural research areas both now and in the near future because of their capacity to streamline agricultural procedures and provide substitute answers for issues that require improvement (Galatali *et al.*, 2021; Liakos *et al.*, 2018) [13, 24].

Numerous studies on a wide range of topics, including breeding, plant production planning, plant classification, yield estimation, plant disease, pest, and weed detection, irrigation management, crop rotation determination, fertiliser and equipment selection, and disease detection, have been conducted by researchers using artificial intelligence techniques in various agricultural fields (Spanaki *et al*., 2022; Dengel, 2013; Orchi *et al.*, 2021) [47, 10, 34].

6. Importance of Mutation Breeding In Horticultural Research

One important breeding strategy for producing variation in horticultural crops is mutation. Features including dwarf plants, earliness, tolerance, and resistance to pests and diseases can all be swiftly enhanced with this technique. Crop breeding and genetics have been transformed by mutation detection and genotypic selection. Breeding for genetic variation or multiplication can be accelerated by induced mutation. In addition to preserving industrial success, it encourages the creation of commercial cultivars to attain nutritional security and provide income for the growers. The significance of mutant breeding in horticulture research is examined in more detail below:

- **Accelerating Genetic Variation:** A technique for creating genetic variation—which is necessary for crop improvement—is mutation breeding. It entails altering plant DNA by applying chemical or physical mutagens. A greater variety of features to choose from can result from this process, which can produce new alleles (gene variations) and even new genes.
- **Developing Desired Traits:** Varieties with enhanced qualities, such as increased yields, enhanced quality, resistance to pests and diseases, and environmental adaptation, can be created by mutation breeding. It can be used, for instance, to produce disease-resistant vegetables, dwarf fruit tree variants, or decorative plants with unusual flower colours.
- **Breaking Linkages:** Mutation breeding can help break the linkage between desirable and undesirable traits, allowing for the selection of plants with only the desired characteristics.This can be particularly useful when breeding for complex traits, where many genes are involved.
- **Overcoming Breeding Barriers:** Mutation breeding can overcome some of the limitations of traditional breeding methods, such as self-incompatibility in fruit crops. It can also be used to induce male sterility, which can be useful for hybrid breeding programs.
- **Cost-Effectiveness:** Mutation breeding can be a cost-effective method for developing improved varieties, especially when compared to methods like gene editing or genetic engineering.It can also be faster than traditional breeding methods, as it can directly induce mutations rather than waiting for natural variations to arise.
- **Generating New Varieties:** Many commercially available fruit, vegetable, and ornamental plant types have been developed globally as a result of mutation breeding. The success of mutant breeding is demonstrated by the emergence of disease-resistant vegetables, miniature fruit trees, and novel floral colours.

Mutation breeding is essentially a potent technique in horticulture research that can assist breeders in creating new and improved varieties more rapidly and effectively, which will ultimately result in horticultural crops that are more diverse and of higher quality.

7. Conclusion

Mutations are crucial to the development of crops. Mutations are rarely a stand-alone breeding technique. They are typically employed following hybrid breeding or through selection breeding. A character that stays the same over many generations can suddenly change through mutation and be passed on to succeeding generations in a different form. Changes in the number of chromosomes and chromosome components are necessary for the mutation to be persistent in plants. These techniques, which significantly aid in the creation of new horticulture breeds and varieties, will be useful in the future for creating new processes using contemporary biotechnology techniques. Therefore, the following are the main benefits of employing mutation approaches in plant breeding: Breeding time is cut in half when a desired mutant free of side effects is discovered. The severe regulations that transgenic plants must adhere to, as well as the duty to notify consumers, do not apply to the kinds created by mutation breeding. In order to meet the demands of consumers and producers, it is imperative that the breeding period be shortened and that new kinds be swiftly introduced to the market. Another crucial element that will influence the consumer's decision is the fact that it is not transgenic. Aside from the unique traits of the varieties created through mutation breeding, their contributions to international peace by guaranteeing the peace and prosperity of nations and to global food security by enabling the production of enough food despite population growth are undoubtedly significant.

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