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Review

Mutation Breeding in Horticultural Plant Species

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Academic Editors: Pedro Martinez-Gomez and Yuri Shavrukov

OBM Genetics
2023, volume 7, issue 4
doi:10.21926/obm.genet.2304198

Received: July 27, 2023
Accepted: October 15, 2023
Published: October 19, 2023

Abstract

In the face of rapid population growth worldwide, humanity's need for plant and animal products is increasing. In this regard, the organizations in charge of production make production and consumption forecasts for the future and try to accelerate the work aimed at increasing production. There are various ways of increasing production. The first of these is the improvement of cultivation techniques, the expansion of irrigated agricultural areas, and the effective control of diseases and pests. The second is to find and produce high-yielding new varieties with appropriate breeding methods. Mutations can be used directly and indirectly in plant breeding. The use of mutations in direct plant breeding gains importance when improving one or two characteristics of a variety with good adaptability is desired. The present study discusses the importance, advantages, and disadvantages of mutation breeding in horticultural plants. It evaluates future mutation breeding and new biotechnological approaches.

Keywords

Horticultural plants; mutation; plant biotechnology; plant breeding

1. Introduction

Breeding, which we also call the process of editing the genetic structure of plants for foreseen purposes or selecting forms that differ genetically, is the procedure of genetically editing plants or developing plants suitable for our goal by using existing genetic variations [1, 2]. Today, breeding studies are carried out on many horticultural plant species with traditional and current biotechnological approaches (Figure 1).

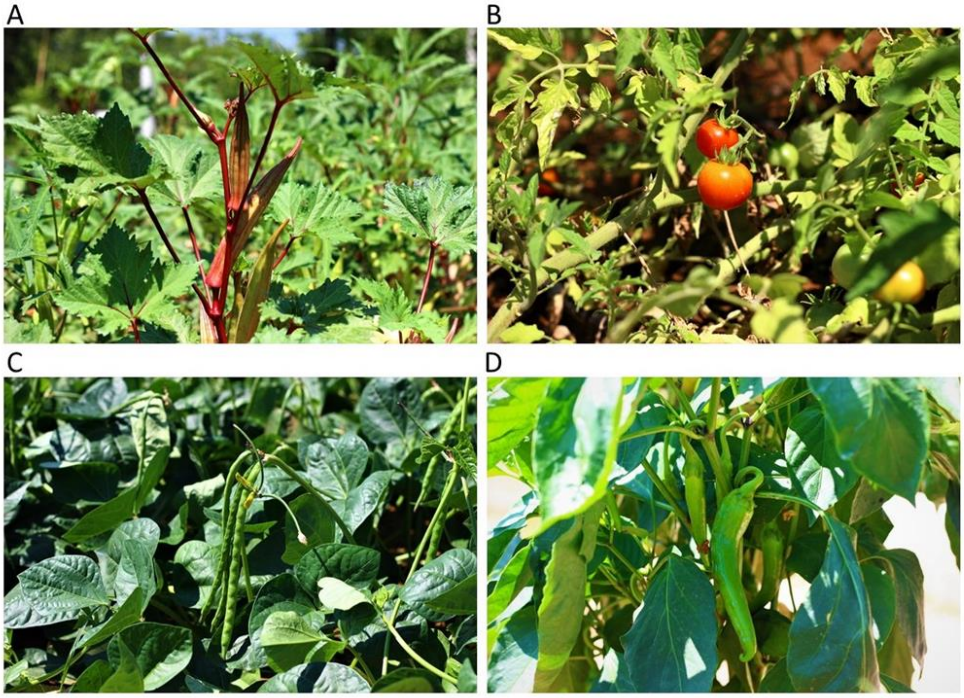

Figure 1 Muğla Metropolitan Municipality, Department of Agricultural Services, Local Seed Bank (Muğla, Turkey), some local horticultural plant cultivars bred in the Collection Garden; **A.** *Abelmoschus esculentus* L. cv. "Purple Okra", **B.** *Solanum lycopersicum* L. cv. "Cherry", **C.** *Vigna unguiculata* (L.) Walp. cv. "Karagöz", **D.** *Capsicum annuum* L. cv. "Village Pepper".

Natural mutations, hybridizations, and selections constitute plant evolution's basis. The history of plant breeding is as old as the beginning of agriculture. Plant breeding was started by humans thousands of years ago. In the pre-agricultural period, people learned to plant seeds in the soil at a particular time of the year. This led to the beginning of the cultivation of plants and the first production of plants. Cultivated plants have been developed using natural variation from different genetic resources and classical breeding methods (selection, hybridization, etc.) [3, 4].

Many genetic techniques, such as selection or genetic engineering, can be alternatives to the natural transfer of genes. Developing pesticide-resistant products, gaining genes that provide resistance against viral diseases with the help of virus coat proteins, and accelerating the nutrient level of cultivated plants are some of the achievements of transgenic technology [5-7].

The element that must be present in plant breeding is the richness of genetic forms in the population. This is called genetic variation, for short. The breeder can easily benefit if this genetic variation is sufficiently present in the existing population. If there is no or not enough genetic variation in the people to be studied, the breeder must take some actions to create this variation [8-10].

Today's modern breeding studies involve creating variation in desired genotypes, making selection through selection, observing, and multiplying. Plant breeders combine several techniques to increase efficiency and shorten the time required for breeding work. As a result of the crossing process to be carried out between the parents in the population, different plants can be obtained, or "Genetic Variation" can be obtained by creating mutations in the individuals forming the population [11, 12].

2. Mutation Breeding

One of the methods of creating genetic variation is "mutation". All changes in the genomic structure are called mutations. Mutations are defined as hereditary changes in the DNA sequence that are not caused by genetic expansion or recombination but result from changes in the physical and chemical structure of the hereditary material (Figure 2) [11, 13].

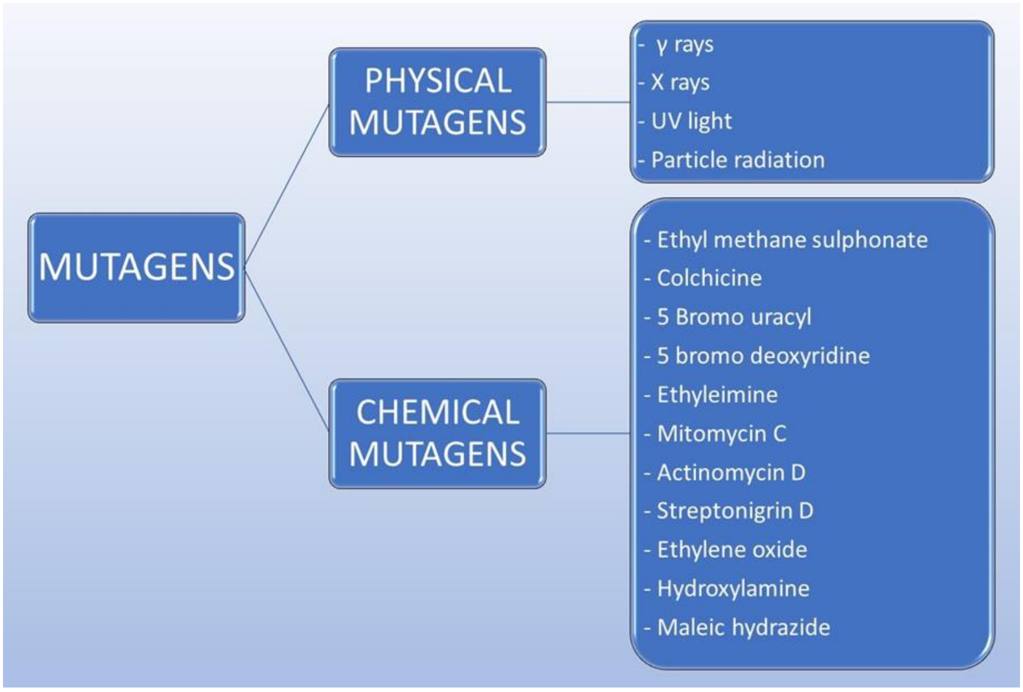

Figure 2 Some physical and chemical agents that cause mutation [14, 15].

The formation of new types in plants and animals through mutation and the idea of benefiting from these mutant types for the first time was introduced in 1901 by a researcher named Hugo de Vries in his work called Mutation Theory. In the following years, plant breeders started to use induced mutations to change plant characteristics [16-18].

Priority strategy in mutation-based breeding studies is the development of new varieties with better characteristics by changing one or more attributes of an accepted type without changing the accepted elements (Table 1). When mutation breeding is compared with hybrid breeding, very little change occurs in the genotype of the variety as a result of mutation breeding. Mutation breeding partially eliminates the long-term requirement in classical cross-breeding studies [13, 19].

Table 1 The significant studies on mutation breeding in the last ten years.

Species	Aims of the study	Technique	Reference
Solanum lycopersicum L. (Tomato)	To improve the variety	Chemical mutagenesis (Sodium azide)	[20]
Solanum lycopersicum L. (Tomato)	For resistance to *Orobanche ramosa* L	Chemical mutagenesis (Ethyl methane sulfonate)	[21]
Solanum lycopersicum L. (Tomato)	For functional genomics studies	Physical mutagenesis (Gamma-ray irradiation)	[22]
Citrus × *sinensis* (L.) Osbeck. (Sweet Orange)	For huanglongbing resistance	Agrobacterium-mediated epicotyl transformation	[23]
Fragaria vesca L. (Strawberry)	For flowering control	Agrobacterium-mediated leaf disk	[24]
Solanum tuberosum L. (Potato)	To develop starch quality	PEG-mediated protoplast transfection	[25]
Oryza sativa L. (Rice)	To develop grain length and width	CRISPR/Cas gene-editing	[26]
Zea mays L. (Maize)	To develop seed color	CRISPR/Cas gene-editing	[27]
Musa spp. (Banana)	To develop a long shelf life	CRISPR/Cas gene-editing	[28]
Ipomoea batatas (L.) Lam. (Sweet potato)	To develop low amylose content	CRISPR/Cas gene-editing	[29]

The breeder's aim in introducing new varieties is to find types with high yield and quality suitable for large areas' climate and soil conditions or to improve the deficient aspects of the existing types. For this purpose, breeders benefit from the natural variations and new techniques and methods they have developed. With conventional breeding methods, which are one of these new techniques and methods, many new practical varieties have been put into the service of agriculture. Variations created by these conventional breeding methods often require a long time, a lot of effort, and a lot of money. The mutation breeding method has started to be used as a new method to save the breeder's time, make a planned study, and obtain new varieties in a short time [29-31].

2.1 Mutation Types

The change of genetic material is generally called "Mutation" and the type that occurs as a result of this is called "Mutant Type". The change can be seen in a gene locus, chromosome structures, and numbers. Mutations are examined in three main groups [32, 33].

2.1.1 Gene Mutations

It is formed by the sequencing of nucleotides and codons, where there is a change in genetic material that produces a specific genotypic appearance or phenotypic difference. Any change in this sequence is called a gene mutation. Such mutations usually occur during replication. If a mutation occurs in a gene in a cell, this mutation can be transferred to all cells formed by the division of this cell. Point mutations can take the form of substitutions, insertions, and deletions. Gene mutations include polyploids and aneuploids [34, 35].

2.1.2 Chromosome Mutations

Chromosome mutations are changes in genetic material that are larger than point mutations. Chromosomal changes are primarily the result of breaks in chromosomes that occur spontaneously or by mutagens. Such mutations can change the location of the gene on the chromosome. It can also increase or decrease the number of genes. Chromosome mutations are examined in four groups: deletion and deficiency, duplication, translocation, and inversion. While the change in deletion, repetition, and inversion is limited to one chromosome, there may be displacement in two or more chromosomes in translocation. There are two types of chromosomal inversion mutation: pericentric (involved in the centromere) and paracentric (in the absence of the centromere) [36, 37].

2.1.3 Extranuclear Mutations

Hereditary factors related to cytoplasm come into play here. Cytoplasmic inheritance is divided into plasmon and plastid estates. Mutated plastids are mainly transmitted from generation to generation via egg cells. Since plastids and microdynes contain DNA, possible mutations in plastid DNA will, in principle, not differ from mutations in core genes. The biochemistry of plasmon mutations is not yet understood. Mutation studies are carried out on cytoplasmic male sterility, and its mechanism is being investigated. More information and research are needed to elucidate the nature and effects of mutagens on cytoplasmic inheritance and elicit beneficial changes [38-40].

3. Mutation Techniques in Horticultural Plants

The objectives of a successful mutation breeding program are to improve the cultivar or even one or a few characters, to create a recognizable morphological marker for promising or even cultivar registration, to restore male sterility or fertility for the production of usable hybrid varieties, and to obtain mutations that are simple to inherit [34, 41].

In mutation breeding studies, the ability of the mutagen to create mutations, the amount of material to be applied and the availability of the mutagen in question are essential. Many physical and chemical mutagens are used to obtain genetic diversity for mutation breeding studies. Physical mutagens: X-rays, gamma-rays, neutrons, beta cathode rays, alpha particle, and protons. These mutagens are divided into two as slow or fast ionizing. Slowly ionizing: ultraviolet radiation, X-rays, gamma rays from radioactive isotopes such as Cobalt-60 or Cesium-137. Fast ionizers are basic or slow neutrons and B particles. These mutagens cause enormous changes in the genetic structure of plant material. These changes cause chromosome breaks and reduce the plant's chance of survival [13, 16].

Chemical mutagens are: Diethyl sulfate, ethyl methane sulfate, methyl methanesulfate, ethylenimemine, N-nitrose N-ethylurea, azides. Chemical mutagens are generally preferred because they are suitable for generating micromutations [14, 15].

Another mutation tool is transposable elements. Transposable genetic elements contain a wide array of DNA sequences, all of which can migrate to new regions in genomes either directly by a cut-and-paste mechanism (transposons) or indirectly via an RNA intermediate (retrotransposons). First discovered in maize plants by geneticist Barbara McClintock in the mid-1940s, it was initially considered a genetic oddity. A few decades later, TEs acquired the anthropomorphic labels "selfish" and "parasitic" because of their replicative autonomy and potential for genetic disruption. Transposons, retrotransposons, T-DNA, retroviruses are important transposable mutagens [13, 33].

3.1 Selection of Breeding Variety

In selecting the main variety, mutation breeding, mixing, and reducing foreign pollination should be done simultaneously. It may also be a newly registered variety, a promising line to be written, an introduction variety, or a good line that has been withdrawn from registration due to a lack of specific characteristics. These features are cracking, color change, winter and summer characteristics such as earliness or late maturity, and short and tall plant characteristics [41, 42].

3.2 Planning the M_1 Generation

In mutation breeding studies, the dose limit to be used in greenhouse, laboratory, and field trials should be determined first. In field trials, it is necessary to grow it in the control and mutagen-treated populations. It allows comparison of treatment effects on control populations, germination, growth, survival, damage, and sterility in M1, as well as eliminating phenotypic change in the parent variety [43, 44].

In mutagen application, it is recommended to use at least three doses and two different mutagens, excluding control. 20% more or 20% less of the 50% growth-reducing dose found in greenhouse and laboratory conditions are the dose limits that can be used in mutation breeding. Amounts that provide a 15–30% growth reduction in ionizing radiation and a 10–30% growth reduction in chemical mutagens are used. The size of the M1 population should be large enough to provide mutations at the expected frequency [44, 45].

In the sowing of seeds in the M1 generation, field preparation, planting time, ambient temperature, weed control should be done carefully. It is necessary to make timely observations to determine the effects of mutagens on the M1 generation. Starting with the M2 generation, selection should be made following the purpose. Applying the pedigree method to M2, M3, and M4 generations is appropriate. Those that maintain their inheritance and show superiority in terms of desired characteristics are taken into yield trials or used as rootstocks in hybridization after they are shown to settle. In this context, it is critical for these studies that all the procedures applied are carried out with great care and effort. For this reason, the data obtained at all treatment stages must be recorded, analyzed, and finalized with appropriate methods [43, 44].

3.3 A Current Approach in Plant Breeding: CRISPR/Cas9

The CRISPR/Cas9 gene editing method has been used in some plant species for various features, especially yield improvement and biotic and abiotic stress management. Most of these studies are considered proof-of-concept studies, as they describe the application of the CRISPR/Cas9 system by disabling specific genes that play an essential role in tolerance mechanisms to abiotic or biotic stress. The biotic stress exerted by pathogenic microorganisms poses severe difficulties in developing disease-resistant plants, accounting for nearly half of the potential yield loss and causing global declines in food production. CRISPR/Cas9-based genome editing has increased resistance to plant diseases and improved tolerance to major abiotic stresses such as drought and salinity [46-48].

New breeding techniques allow scientists to add desired traits more precisely and quickly than in conventional breeding. CRISPR/Cas9-based genome editing technology has had a revolutionary impact on plant research and breeding. Recently, it has been applied to improve critical agricultural properties, such as functional studies and combating biotic and abiotic stresses in many plant species. However, although the CRISPR/Cas9 vector platform has been established and its efficacy in genome editing has been tested, the potential of the CRISPR/Cas9 system has not been fully explored. Although various modifications to this technology have led to targeted productivity gains, most of the work done is preliminary and needs improvement. In the future, genome editing technology with CRISPR/Cas9 will be an essential field of study to apply in plant breeding to increase yield, nutritional value, disease resistance, and other agronomic traits [49, 50].

4. Artificial Intelligence Technologies in Plant Breeding

Artificial intelligence is about imitating natural systems, such as humans and animals, using man-made tools such as computers and robots. This method involves understanding how information can be represented so that it can be stored in computer memory and inferences can be made from that information automatically. It also includes understanding how decisions can be made, action plans created based on stored information, and how computerizable information can be obtained by learning from sample data or questioning human experts. Artificial intelligence systems are machines developed with human intelligence abilities such as information acquisition, perception, learning, thinking and decision making. They examine and formulate the mental functions related to intelligence in humans with computer models and apply them to different systems [51-53].

The primary goal of agricultural production is to provide economic, sustainable, and productive management in plant and animal production. For this purpose, alternative solutions are developed for facilitating agricultural operations and problems requiring solution or improvement by using technology in various subjects such as increasing productivity and product quality in agriculture, using minimum inputs, food safety, protecting natural resources and the environment. During the development period of agricultural production, due to the rapid development in information technologies after mechanization, automation, control, and informatics, smart machines and production systems that control devices have begun to replace traditional production methods. Information technologies consist of hardware, algorithms, and software developed to manage the processes of obtaining, processing, storing, transferring, and using information. As a result of evaluating the existing knowledge and experience in agriculture together with machine learning, deep learning, artificial intelligence, modeling, and simulation applications offered by information technologies, it has been possible to realize applications that require precision, such as breeding easily. Artificial intelligence applications are expected to take place in the most important agricultural research topics today and shortly due to their potential for facilitating agricultural processes and developing alternative solutions to problems that need solution or improvement [54, 55].

By using artificial intelligence techniques in many fields of agriculture, many studies have been carried out by researchers on many subjects such as breeding, plant production planning, classification of plants, yield estimation, detection of plant diseases, pests, and weeds, irrigation management, determination of crop rotation, selection of the most suitable fertilizer and equipment, and detection of animal diseases [54, 56-58].

5. Conclusions

Mutations play an essential role in the formation of cultivars. Mutations are rarely a breeding method that can be used alone. They are generally used through selection breeding or after hybrid breeding. A character that remains constant for generations undergoes a sudden change with mutation and can be passed on to new generations in its changed form. For the mutation to be permanent in plants, there must be changes in the number of chromosomes and parts on the chromosome. That is, the mutation must occur at the growth points of the plant's shoots. These methods, which make significant contributions to the development of new horticultural varieties and breeds, will be beneficial in developing new procedures with current biotechnology approaches in the future. As a result, the most important advantages of using mutation techniques in plant breeding are as follows: When a desired mutant free of side effects is found, the breeding time is reduced by approximately half. The varieties developed due to mutation breeding are exempt from the restrictive laws that transgenic plants are obliged to comply with and the obligation to inform the consumer. It is essential to shorten the time spent in the breeding phase and meet the consumers' needs and the producers' competition by introducing new varieties to the market quickly. The fact that it is not transgenic is also an essential factor that will affect the consumer's choice. Apart from specific characteristics of the varieties developed as a result of mutation breeding, their contribution to global food security by making it possible to produce sufficient food despite the increasing population and their contribution to international peace by ensuring the peace and prosperity of countries has an undeniably significant impact.

Acknowledgments

We would like to thank Muğla Metropolitan Municipality, Agricultural Services Department Employees for all their support.

Author Contributions

All authors contribute equally.

Competing Interests

The authors have declared that no competing interests exist.

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