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Poonam Maurya
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Bhupendra Sagore
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Shikha Jain
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Shikha Saini
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Aditya Ingole
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Rakesh Meena
Ph.D. Research Scholar, Department of Fruits Science, College of Horticulture and Forestry, Jhalarapatan City, Jhalawar, Agriculture University, Kota, Rajasthan, India

Vinay Kumar
M.Sc. Ag, Department of Horticulture (Fruit science), Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Corresponding Author
Bhupendra Sagore
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Mutational breeding in fruit crops: A review

Poonam Maurya, Bhupendra Sagore, Shikha Jain, Shikha Saini, Aditya Ingole, Rakesh Meena and Vinay Kumar

Abstract
Fruits play a significant role in human nutrition and as a commercial item in commerce; consequently, fruit crop genetic modification is required to increase production. Fruit crop improvement though traditional means is impeded by a prolonged long juvenile phase, incompatibility, excessive fruit drop, polyploidy, apomixes, heterozygosity etc. The use of genetic variation, whether natural or induced, aids tremendously in fruit tree genetic improvement. Mutational breeding is a useful strategy for developing horticultural varieties with better phenotypic and genotypic characteristics. Mutations are the primary cause of genetic differences found in plants. Mutagens, both physical and chemical, are mostly utilized to induce desirable traits. Modification in flowering time and fruit ripening, breaking linkages of undesirable traits, change in fruit colour, inducing dwarfism, inducing self-compatibility, seedlessness, self-thinning and pathogen resistance, increasing ploidy level, inducing variability in already adapted species, restoring fertility in sterile hybrids, increasing fruit size with better taste and aroma have all been successfully introduced through mutation breeding.

Keywords: Mutation, genetic variation, dwarfism, mutagens, seedlessness, and CRISPR/Cas9

Introduction
Mutation is defined as "heritable changes in an organism's genetic material that are not the result of genetic segregation or recombination" (Van Harten, 1998) [21]. Chemical alterations at the gene level are the cause of these modifications. Such alterations may result in new and heritable character variants in plants, which can be identified and used to develop crop varieties with novel traits. Mutation breeding is the process of genetically improving crop plants for various economic characteristics through the application of induced mutations. When desirable variability is not present in cultivated varieties or cultivated species germplasm, or when a high yielding variety has oligogenic faults such as disease susceptibility, this approach of crop development is applied. Furthermore, because there is a tight linkage between desired and unwanted characteristics, mutation breeding is the ideal option. When sexuality is missing and the development of diversity through the recombination process is impossible or the breeding cycle is very prolonged, such as in most fruit crops, mutation breeding can assist in the creation of new variability. Other obstacles (such as polyploidy, apomixes and incompatibility, etc) might make obtaining usable recombinants a difficult task (Hansche and Beres, 1980) [5]. Induced mutations, on the other hand, affect only one or a few particular attributes of an elite cultivar and can contribute to fruit enhancement while causing little disruption to the fruit industry or consumers. Mutation induction may be the sole appropriate technique of generating variety for developing new cultivars of crops that do not produce seeds, such as edible bananas or seedless grapes (Pathirana, 2011) [13].

Type of mutations: Mutations can be broadly divided into following two categories-
1. Intragenic or point mutations: occurs within a gene in the DNA sequence and restore function of mutant gene product.
2. Intergenic or structural mutations within chromosomes: chromosome breaks and rearrangements are the cause of structural mutations. It includes inversions, deletions translocations, and duplications. Haploidy, polyploidy and aneuploidy are chromosomal number alterations caused by mutations. Deletions and insertions of one or more base pairs are referred to as deletions and insertions, respectively, and frame-shift mutations occur when they do not occur in multiples of three nucleotides in the sequence. Approximately 90% of chromosome abnormalities caused by ionizing radiation are deletions, which are generally fatal.

Translocations and inversions (180 rotations of nucleotide blocks) are also common in agricultural plant evolution.

Changes in chromosome number caused by mutations at the genome level are also essential in crop evolution and plant breeding. Allopolyploidy, autopolyploidy, and aneuploidy are the three types of mutations. Allopolyploidy occurs when the genomes of two or more species are combined, and it is most typically caused through interspecific or intergeneric hybridization followed by chromosome doubling. Autopolyploidy occurs when the number of chromosomes within a species is multiplied, and the nucleus contains more than two homologous chromosomes. Autopolyploidy can be generated by using agents that prevent the production of spindle fibres during mitosis, allowing the divided chromosomes to remain in the same cell (Pathirana, 2011) [13]. The most often used compounds for artificial polyploidization are colchicine and oryzalin.

Based on cause of mutation: Mutation can be broadly divided into two categories-

1. Spontaneous mutation: At a rate of 10^{-5}-10^{-8}, spontaneous mutations in agricultural plants occur spontaneously throughout adaptations and evolutionary processes. This frequency is insufficient for inducing variations in a crop's genetic architecture in order to increase desired traits (Zakir, 2018) [23]. In terms of the time of occurrence and the gene in which they occur, spontaneously occurring mutations are extremely rare and random events. Many of the mutations may be harmful, rendering the organism less adapted to its environment, and some may even be fatal, but they can sometimes aid in the production of a diverse variety of useful recombinant genotypes in successive generations (Franks *et al.*, 2002) [3].

Bud mutations are a valuable source of variability, as they can lead to variations with desirable traits like as superior fruit quality (Karanjalker *et al.*, 2016) [8]. Color changes in the red or purple anthocyanin content of fruit are the most prevalent and widespread mutations among bud sports (Walker *et al.*, 2006). Bud sport, unlike traditional hybridization breeding, is caused by genetic variation in somatic cells, which results in qualitative and quantitative phenotypic changes in plants. It may be seen in many vegetatively propagating plants, including grapes (Liu *et al.*, 2007).

Table 1: Identified spontaneous mutants in fruit crops

Fruit crop	Original variety	Mutant variety	Nature of mutation	Traits
Mango	Rosado de Lea Davis haven	Rosica Haden	Bud sports	Precocious, regular bearer, and larger fruit size Large fruit size
Mandarin	Owari Pongan	Clausellina Pongan 86-1	Bud sport	-
Navel Orange	Bahia Washington	Baianinha Navelina, Navelate, Marrs, Leng, Autumn Gold, Powell Summer, Winter Red	Limb sports	-
banana	Highgate Motta Poovan	Gros michel Poovan	Sports	Semi dwarf
Pear	Clapp's Favourite	Starkrimson	Bud sports	Spotting of coloured
Grapefruit	Foster	Hudson	Bud sports	Deep red flesh

Ray, 2002 [15]

2. Induced mutation: It occurs when mutation is purposely induced by various mutagenic agents (physical or chemical). Induced mutations are a powerful tool for improving natural genetic resources, and they have aided in the development of better fruit varieties. It can be used to create genetic variations without affecting a genotype's valuable features. Physical and chemical mutagens are used to carry out this mutagenesis. In 1930, Stadler and Murneek used X-rays to treat apple scions, which was one of the first attempts to induce mutations. Disease resistance (in Japanese pear and peach), reduced height (in pomegranate, papaya), seedlessness (in guava, citrus), and earliness (in banana, apple, apricot, jujube and plum) are some of the important agronomic properties improved by induced mutations in certain fruits crops.

Mutagens

A mutagen is a natural or man-made agent that causes genetic mutation in plants or organisms, such as radiation or a chemical compound. Ionizing radiations and chemical mutagens are the most commonly employed mutagens (Kamatyanatt *et al.*, 2021) [7]. All sorts of planting materials, such as complete plants, generally seedlings, and *in vitro* cultivated cells, can be used for mutagenesis. Nonetheless, seed is the most often used plant item (Oladosu *et al.*, 2016) [12]. Mutagens are divided into two categories: Physical mutagens and chemical mutagens (Mba *et al.*, 2010; Shu *et al.*, 2012) [10, 18].

1. **Physical mutagens:** Electromagnetic radiation, such as gamma rays [emitted from radioactive cobalt (^{60}Co)], X-rays, and UV light, as well as particle radiation, such as fast and thermal neutrons, α and β particles, are examples of physical mutagens. Ionizing radiation causes chromosomal breakages, permitting cross-linking of DNA strands, nucleotide deletion, and nucleotide substitution (Oladosu *et al.*, 2016) [12]. UV rays are a non-ionizing physical mutagen that has been used to irradiate cell suspensions and pollen grains in the late or early uninucleate phases due to their low penetration.

Ionizing radiation can penetrate deeper into tissue and cause a variety of molecular alterations. X-rays and gamma-rays have been the most extensively employed ionizing radiation use for fruit crop improvement (Mohammad, 2001; Beyaz and Yildiz, 2017) [11, 2]. The use of gamma irradiation to create genetically stable and disease-free mutants is a helpful approach because gamma rays have a shorter wavelength than X-rays and neutrons and they can penetrate deeper into tissues (Amano, 2006) [1]. The highest mutation rates will be seen in doses that cause 25 to 50 % lethality (LD_{25}-LD_{50}) in mutant plants.

Table 2: Physical mutagens used for inducing mutation

Mutagens	Sources	Characteristics	Hazards
Gamma rays	Radioisotopes and nuclear reaction	Radioisotopes and nuclear reactors create electromagnetic radiation that is particularly penetrating into tissues; sources include ^{60}Co (Cobalt-60) and ^{137}Cs (Caesium-137)	Dangerous, very penetrating
X-rays	X-ray machine	Electromagnetic radiation (EMR); penetrates tissues from a few millimetres to several centimetres.	Dangerous, penetrating
Alpha particles	Radioisotopes	Obtained from radioisotopes; a helium nucleus capable of heavy ionization; very shallowly penetrating	Very dangerous
Beta particles	Radioactive isotopes or accelerators	Produced in particle accelerators or from radioisotopes; electrons; ionize; shallowly penetrating; sources contain ^{32}P and ^{14}C	May be dangerous
Ion beam	Particle accelerators	Positively charged ions are produced and accelerated to a high speed (about 20% to 80% of the speed of light) to deposit a large amount of energy on a target.	Dangerous
Neutrons	Nuclear reactors or accelerators	There are several forms (fast, slow, and thermal); they are created in nuclear reactors; they are uncharged particles that penetrate tissues to depths of several centimeters; and their source is ^{235}U.	Very hazardous
Protons	Nuclear reactors or accelerators	Produced in nuclear reactors and accelerators; derived from the hydrogen nucleus; capable of penetrating tissues up to several centimeters	Very dangerous

Oladosu *et al.,* 2016 [12]

2. **Chemical mutagens:** Chemical mutagens are alkylating chemicals that can be used to induce mutations. Chemical mutations are more consistent and specific to a single gene than physical mutations and include Diethyl sulfate (DES), Ethylenimine (EI), Ethyl methane sulfonate (EMS), Sodium azide, and Colchicine. Colchicine is most commonly and commercially used agent for polyploidization due to its consistency and efficacy. It is a type of alkaloid found in meadow saffron (*Colchicum autumnale* L.). Colchicine is used to make tetraploids by preventing chromosomal segregation during metaphase in meiosis, effectively doubling the number of chromosomes and ploidy. The generation of residuals is a key issue with chemical mutagens. Chemical LD_{50} is calculated by adjusting the treatment concentration and duration, the solvent employed (e.g. Dimethyl sulfoxide (DMSO), or the pH of the solution (Lamo *et al.*, 2017) [9].

EMS has emerged as the mutagen of choice for creating mutant populations for high-throughput screening, such as TILLING populations (Pathirana, 2011) [13]. These chemical residues have the potential to cause cancer and other health issues in humans. Consequently, despite the encouraging outcomes of chemical plant mutagens, researchers have been trying other alternatives such as physical and biological mutagens, which leave no residues after treatment (Udage, 2021) [20]. Chemical mutagens have a high reactivity. It is necessary to use chemicals from new batches.

Table 3: Chemicals used for inducing mutation

Mutagen group	Example	Mode of action
Alkylating agents	1-methyl-1-nitrosourea (MNU); 1-ethyl-1-nitrosourea (ENU); methyl methanesulphonate (MMS); ethyl methanesulphonate (EMS); dimethyl sulphate (DMS); diethyl sulphate (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)	Add methyl or ethyl groups to bases, and the alkylated base may degrade to generate an a basic site, which is mutagenic and recombinogenic, or mispair to result in mutations during DNA replication, depending on the affected atom.
Azide	Sodium azide	Similar as alkylating agents
Hydroxylamine	Hydroxylamine	Similar as alkylating agents
Antibiotics	Actinomycin D; streptonigrin; mitomycin C; azaserine	Male sterility has also been linked to chromosomal abnormalities.
Nitrous acid	Nitrous acid	Deamination occurs when cytosine is replaced by uracil, which can combine with adenine and lead to transitions in following replication cycles.
Acridines	Acridines orange	Intercalate between DNA bases, causing a distortion of the DNA double helix, which the DNA polymerase interprets as an extra base and inserts an extra base opposite the stretched (intercalated) molecule. Frame shifts, or changes in the reading frame, arise as a result of this.
Base analogues	5-bromouracil (5-BU); 5- bromodeoxyuridine; 2-aminopurine (2AP); maleic hydrazide	During DNA replication, incorporate into DNA in place of the usual bases, generating 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)

***In vitro* mutagenesis**

The combination of *in vitro* procedures and mutation breeding has resulted in the emergence of numerous novel types. This combined strategy generates a new genomic variation. *In vitro* techniques for mutation induction are important because they allow for the induction of mutations in a large number of propagules in a small space, and several cycles of subculture can be carried out in a short time to dissociate mutated sectors from non-mutated ones, and increase the population to large enough numbers for selection. It improves the efficiency of acquiring desired genotype variation, selection, and multiplication. A single mutagenesis therapy is *in vitro*

mutagenesis. It is possible to create disease-free planting material. Citrus DG-2 somaclone has evolved to be resistant to canker disease. In *Citrus jambhiri*, crude culture filtrate was used as a selective agent to *in vitro* select *Phytophthora parasitica* resistant lines, which were then screened by randomly amplified polymorphic DNA analysis (Savita *et al.*, 2017) [17]. Huang long bing (HLB) disease resistance in *Citrus nobilis* embryo culture exposed with gamma rays (Purba and Priskila, 2015) [14].

Table 4: Achievements in *in vitro* mutation breeding in vegetatively propagated fruit crops

Fruit Crop Species	Treated Material	Mutagen and Dose (LD 50)	Plant Regeneration	Outcome
Banana (*Musa* spp.)	Shoot tips	Carbon-ion beam (0.5-128 Gy)	Direct regeneration	Disease-resistant lines
Banana (*Musa* spp.)	Shoot tips	γ-rays (60 Gy)	Direct regeneration	Mutant Novaria; earliness
Banana var. Lakatan	Shoot tips	γ-rays (40 Gy)	Direct regeneration	Mutant variety Klue Hom Thong KU1
Pineapple var. Queen	Crowns	γ-rays	Axillary bud regeneration	Lines with reduced spines
Pear	*In vitro* shoots	γ-rays (3.5 Gy)	Micro-cuttings from shoots	Mutants for russeting, fruit shape and size, small tree size, wide branch angle and short internodes

Suprasanna *et al*., 2015 [19]

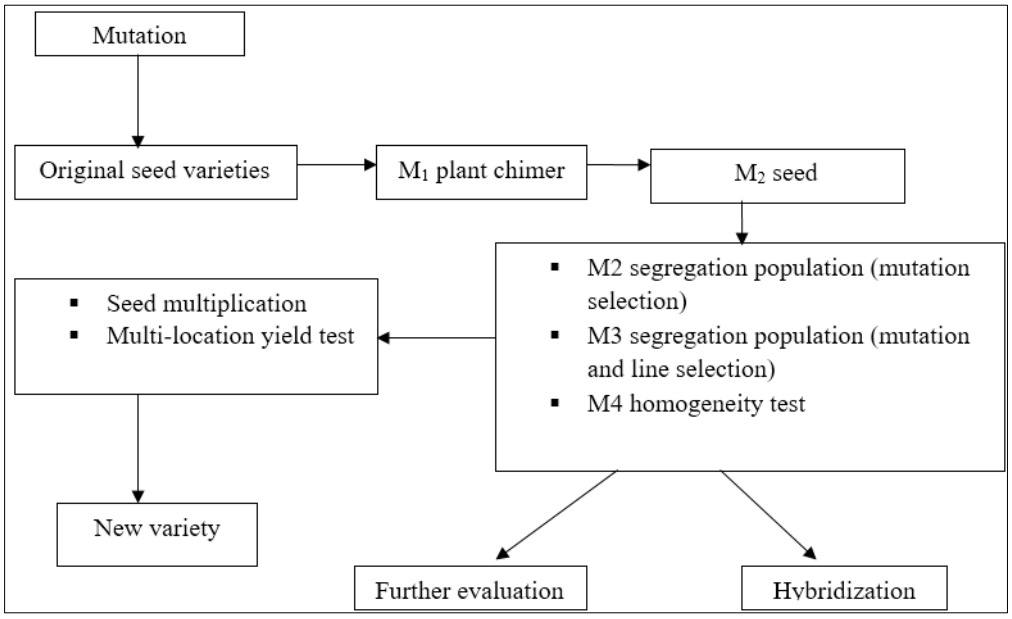
CRISPR/Cas9-induced mutation

The new breeding technique known as clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein-9 (Cas9) has the potential to improve many traits rapidly and efficiently, such as quality, yield, disease resistance, abiotic stress tolerance, and nutritional aspects in fruit crops (Hua *et al*., 2017) [6]. Since this technique was initially used for plant genetic engineering in 2013, several attempts have been made to make it a more powerful tool, for example, to enable precisely targeted DNA mutations or genetic alterations. Site-directed mutagenesis using genetic approaches can provide a wealth of resources for fruit crop breeding. Because of its simple operation and high mutation efficiency, this system has been applied to obtain new germplasm resources via gene-directed mutation (Wan *et al*., 2021) [22]. Precise genome editing requires accurate and specific gene function regulation. Mutations in gene exons can alter protein function, and mutations in exon-intron splice regions can result in various alternative splicing variants. With the availability of whole-genome sequencing data, and information about gene function for important traits, CRISPR-Cas9 editing to precisely mutate key genes can rapidly generate new germplasm resources for the improvement of important agronomic traits. Bioactive chemicals such as anthocyanins, malate, aminobutyric acid, and lycopene can be improved by this technique. It can also enrich nutrients in fruits.

Procedure of mutation breeding

Any mutant breeding approach necessitates a series of steps. Mutation induction is the initial phase, which involves exposing plant propagules to a physical, chemical, or biological mutagen (Suprasanna *et al.*, 2015) [19]. The procedure of picking desired individuals from a huge population of treated mutants is called mutant screening in the second stage. Seeds directly exposed to mutagen during the mutation breeding process are known as the M_0 generation, which generates M_1 plants upon germination. The M_2 generation is the result of self-fertilization of M_1 generation crops. Cross pollination among the M_1 population should be avoided at all costs, since this will result in the development of new variation that will be difficult to distinguish from the effect of mutation. M_1 mutant plants are heterozygous genetically. This is because during treatment, a single mutation affects only one allele. Only dominant mutations can be detected in M_1, and recessive mutation expression cannot be detected at this time. In the M_2 generation, screening and selection begin.

The process of determining whether a selected individual is a true mutant or a false mutant is known as mutant confirmation or mutant verification. Re-evaluating putative mutations under replicated and extensive conditions with a larger sample size can help with this (Shu *et al.*, 2012) [18]. Mutant variants are screened and desired mutants are chosen based on their phenotypic traits. Phenotypic selection is more labour intensive and specialized than genotypic selection (Jankowicz-Cieslak *et al.*, 2016). Seed multiplication for extensive field experiments follows when a mutant line looks to have a promising characteristic. The mutant line, the mother cultivar, and other varieties will all be evaluated in this situation. The goal of field trials is to see if the mutant has the potential to become a commercial variety that outperforms the mother cultivar.



Oladosu *et al.*, 2016 [12]

Fig 1: Basic process involved in mutation breeding

Table 5: Achievements though mutational breeding in fruit crops

Fruit crops	Cultivars released	Mutagens	Year	Country	Improved traits
Almond	Supernova	gamma rays (30 Gy)	1987	Italy	Lateness
Apricot	Early Blenheim	ThN	1970	Canada	Earliness
Apple	Golden Haidegg	Gamma rays (50 Gy)	1986	Austria	Fruit size
	McIntosh 8F-2-32	Gamma rays (50 Gy)	1970	Canada	Skin colour
	Blackjoin BA 2 520	Gamma rays (50Gy)	1970	France	Fruit colour
	Balrene	EMS	1970	France	Earliness
	Lysgolden	Gamma rays (50 Gy)	1970	France	Rust resistant
	Courtavel	gamma rays (50 Gy)	1972	France	Shortness
	Courtagold	gamma rays (50 Gy)	1972	France	Shortness
	Senbatsu-Fuji-2-Kei	Gamma rays (60 GY)	1985	Japan	Fruit colour
	Shamrock	Gamma rays	1986	Canada	Earliness
Banana	Novaria	gamma rays, *in vitro*	1993	Malaysia	Earliness
	Klue Hom Thong KU1	gamma rays, *in vitro*	1985	Thailand	Bunch size
	Fuxuan 01	Gamma rays	2005	China	-
	AL-BEELY	Gamma rays	2007	Sudan	-
	Pirama 1	Gamma rays (30 Gy)	2019	Indonesia	-
Fig	Bol	Gamma rays (50–70 Gy)	1979	Russian Federation	not specified
Grape	Fikreti	gamma rays	1986		Earliness
Indian jujube	Mahong	MNH (0.02–0.04%)	1986	Viet Nam	Fruit morphology
	Dao tien	MNH (0.02–0.04%)	1986	Viet Nam	
Lemon	Eureka 22 INTA	x-rays(10Gy)	1987	Argentina	Fruit set, quality
Grapefruit	Star Ruby	thN	1970	USA	Seedless
	Rio Red	thN	1984	USA	Fruit colour
Loquat	Shiro-mogi	Gamma rays (200 Gy)	1982	Japan	Fruit size
Mandarin	Zhongyu 7	Gamma rays (100 Gy)	1985	China	-
	Zhongyu 8	Gamma rays (100 Gy	1986	China	-
	Hongju 420	Gamma rays (100 Gy	1986	China	-
	NIAB Kinnow	Gamma rays (20 Gy)	2017	Pakistan	Sparse seeded
	PAU Kinnow-1	Gamma rays (30 Gy)	2017	India	seedless
Orange	Xuegan 9-12-1	Gamma rays (100 Gy)	1983	China	seedless
	Hongju 418	Gamma rays (100 Gy)	1983	China	seedless
	Valencia 2 INTA	x-rays (20 Gy)	1987	Argentina	Fruit quality
Papaya	Pusa nanha	Gamma rays (150 Gy)	1987	India	Dwarfness
Peach	Magnif 135	Gamma rays	1968	Argentina	Fruit size
	Plovdiv 6	Gamma rays (10 Gy)	1981	Bulgaria	Yield
	Shaji 1	CO2 laser	1985	China	Fruit quality
Pear	Fuxiangyanghongdli	Gamma rays (2.5 Gy)	1983	China	-
	Gold Nijisseiki	Gamma rays	1993	Japan	Disease resistant
	Kotobuki Shinsui	Gamma rays	1996	Japan	Disease resistant
Plum	Spurdente-Ferco	gamma rays	1988	France	Earliness
Pomegranate	Karabakh	gamma rays (50-70 Gy)	1979	Russian Federation	Not specified

	Khyrda	gamma rays (50-70 Gy)	1979	Russian Federation	Dwarfness
Sour cherry	Plodorodnaya Michurina	x-rays	1977	Russian Federation	Fruit set
	Polukarlik Orlovskoi	gamma rays	1979	Russian Federation	Dwarfness
	Polukarlik Turgenevk	gamma rays	1979	Russian Federation	Dwarfness
	Karlik Samorodka	gamma rays	1979	Russian Federation	Dwarfness
	Nishina Zao (DT2008)	Ion beams	2009	Japan	-
Sweet cherry	Lapins	x-rays	1983	Canada	Larger size, firmer
	Stella	X-rays (50 Gy)	1968	Canada	self-fertile
	Stella 16A-7	X-rays (50 Gy)	1972	Canada	Compact growth
	Compact stella 35B-11	X-rays (40 Gy)	1974	Canada	compact growth
	Sunburst	X-rays (50 Gy)	1983	Canada	Fruit size
	Burlat C1	Gamma rays	1983	Italy	Compact growth
	Nero II C1	Gamma rays	1983	Italy	Compact growth
	Ferrovia spur	X-rays (4 Gy)	1992	Italy	Shortness
	Super 6	Colchicine	1997	Japan	-
	Roman Nishiki	Colchicine	2001	Japan	-
	ALDAMLA	Gamma rays (25 Gy)	2014	Turkey	-
	BURAK	Gamma rays (50 Gy)	2014	Turkey	-

Mohammad, 2001 [11]; Sattar *et al.*, 2021

Detection of mutants

New molecular biological methods that can screen mutants at the gene level have been created in the previous decade. Although genome sequencing is the "gold standard" for mutation identification since it can show the specific position and type of a mutation, it is expensive to apply to large populations and is rarely utilised in practical mutant breeding. Furthermore, direct sequencing might miss heterozygous alleles. As a result, numerous approaches for detecting sequence variations in amplicons in mutant populations have been established. DNA markers related to mutant genes can be utilised for marker-assisted gene selection and tracing. TILLING (Target Induced Local Lesions IN Genome) has evolved as a reliable, high-throughput technology that may be used to indentify point mutation at a specific locus in the mutated gonome (Jankowicz-Cieslak *et al.*, 2017) [4]. Chemical mutagenesis of the whole genome is used to create TILLING populations, which are subsequently detected using an enzymatic technique in combination with gel electrophoresis or denaturing high-performance liquid chromatography. It relies on endonucleases to detect single-base mismatches in heteroduplexes.

Conclusion

In order to create variation in fruit crops, mutation is an important breeding strategy. It allows for the rapid enhancement of traits such as dwarf plant, earliness, tolerance, and resistance to numerous diseases and pests in a short period. Using new technology, mutation identification or selection at the genotypic level has transformed the way mutations are now used in genetics and breeding in fruit crops. *In vitro* culture in combination with induced mutation has been shown to speed up the breeding process for genetic variability or multiplication. It also aids in the development of commercial cultivars in order to meet the nutritional security goal.

Future thrust

More research into directed mutagenesis in vegetative fruit crops is needed. In fruit crops, the effects of combined mutagen treatment and recurring mutagenesis must be explored. The use of markers to detect mutations at an early stage is also required. More research is needed on *in vitro* mutation breeding and CRISPR/Cas-9 approaches, especially for fruit crops.

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