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REVIEW ARTICLE

Recent advances in mutagenesis for commercial fruit crop improvement: A comprehensive review

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Abstract

Mutagenesis, the deliberate induction of genetic alterations, plays a critical role in modern fruit crop improvement. This review explores the impact of mutagenesis methods discussed on diverse fruit crops, highlighting advancements in traits. Such asdeveloping dwarf fruit mutant lines with enhanced resistance to biotic and abiotic stressors, seedlessness, yield and fruit quality have been a major focus recently. Traditional breeding techniques have often led to a genetic bottleneck, limiting. diversity available for crop improvement.To overcome these limitations, plant breeders have adopted innovative approaches like genome editing and mutation. The mutagenesis experiments aim for a 30-80 % survival rate of treated seeds or explants. This balance ensures a high enough mutation load without excessive lethality, which would reduce the population size for selection. *In vitro* random mutagenesis relies on the application of physical and chemical mutagens to increase the frequency of mutations thus accelerating the selection of varieties with important agronomic traits. Mutation breeding consists of three main elements to develop a new trait: mutation induction, selection of desirable mutants through phenotypic screening and genetic characterization using molecular markers. Mutation breeding involves three key steps: mutation induction, phenotypic selection and genetic characterization them using molecular markers (e.g., DNA markers, SNPs) to understand the genetic basis of the observed traits. The physical mutagens are successful; breeders are inclined breeders are given increasingly inclined to use novel genome editing toolslike CRISPR/Cas9 gene editing technology to modify plants. The limitations of random mutagenesis, breeders are increasingly adopting novel genome editing technologies. Tools like Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), RNA interference (RNAi) and especially CRISPR/Cas systems offer several advantages. CRISPR/Cas9 offers precise genome editing capabilities. It enables targeted modifications that enhance desirable traits and address critical challenges in fruit production. This review provides mutagenesis techniques and their applications, emphasizing their importance in developing improved and sustainable fruit cultivars to meet the demands of a growing global population.

Keywords: chemical mutagens; CRISPR/CAS9; crop improvement; genome editing; *in vitro* multiplication; mutagenesis; physical mutagens

Introduction

The global population is expected to reach 10 billion by 2050, but no effective strategies to ensure a sufficient food supply in demand (1). According to the WHO (2), approximately 735 million individuals, representing 9.2 % of the world's population, experienced hunger in 2022. From 2021 to 2023, the Minimum Dietary Energy Requirement (MDER) for all country classifications indicates that an average daily calorie consumption increase of 80 g per day in fruit consumption is associated with an 11 % reduction in the risk of all-cause mortality (3). The demand for such high-quality fruits is being met in part by these plant breeding practices (4). The long juvenile phase and the extensive field space needed for cultivating temperate fruits present significant challenges to traditional breeding methods. Recent advancements in scientific research offer numerous possibilities and innovations in plant breeding and also to overcome these limitations, various molecular biological approaches have been utilized to simplify fruit breeding (5-7). This inherent genetic diversity can lead to unpredictable segregation patterns in the progeny, resulting in a substantial proportion of offspring exhibiting undesirable traits. Furthermore, polyploidy, incompatibility, apomixis and extended juvenile periods pose significant challenges in obtaining desirable recombinants in perennial fruit crops and inhibiting efficiency in conventional breeding programs (8). Mutation breeding offers a promising alternative, utilizing spontaneous, physical (e.g., radiation) and chemical (e.g., ethyl methane sulfonate [EMS], dimethyl sulfate [DMS], colchicine) mutagenesis to induce genetic variation. Mutagenesis has already been utilized in fruit crops to introduce with beneficial features that impact fruit color, flowering time, fruit ripening, plant size, self-compatibility, self-thinning and pathogen resistance that artificial polyploidy induction may help improve the quality of significant fruit crops (9, 10). Therefore, introducing variability into these genotypes for a range of desired properties, including resistance to salt and plant stature, would render them extremely valuable as rootstocks. Induced mutations are frequently used to introduce crops such as mangos, grapes, guava, papaya, plum, citrus and jamun (11-13). However, mutation breeding offers a solution to this challenge, as it allows for the rapid development of a new variety or cultivar with improved characteristics (14).

In vitro culture techniques significantly enhance the efficiency of mutagenesis by expanding the available plant material for treatment (nodal segments, organs, tissues and cells) and *in vivo* buds, facilitating more effective exposure and selection of mutated cells (15). Furthermore, *in vitro* culture enables the handling of large populations for mutagenic treatments, facilitating efficient selection and clonal propagation of desired variants. Additionally, it reduces propagation cycles and ensures high phytosanitary conditions throughout the mutagenesis process (16). Several studies have investigated the radiosensitivity of *in vitro* cultures in various fruit crops. For instance, gamma irradiation of micro-cuttings in Japanese plum (*Prunus salicina* Lindl.) cv. "Shiro" revealed differential tissue responses, providing valuable insights for optimizing mutagenic protocols (17).

This system comprises three key components: catalytically inactive Cas9 (dCas9) fused to a transcriptional effector (activator or repressor), a customizable single-guide RNA (sgRNA) that specifically targets the promoter region of a gene of interest. The binding of the dCas9/sgRNA-effector complex to the target gene promoter exerts transcriptional interference by impeding RNA polymerase progression through mechanisms such as blocking polymerase binding or inhibiting elongation. While CRISPRi and RNA interference (RNAi) both function to suppress gene expression, they employ distinct molecular mechanisms to achieve this outcome (18). In essence, CRISPRi suppresses gene expression at the transcriptional level by inhibiting RNA polymerase activity, whereas RNA interference (RNAi) operates at the post-transcriptional level by mediating mRNA degradation. CRISPR/dCas9 technology has revolutionized functional genomics by providing a powerful and versatile platform for precise transcriptional modulation (19). A modified version of Streptococcus pyogenes Cas9, termed SpCas9-NG, was developed to expand the range of targetable PAM sequences beyond NGG to include NG motifs, thus broadening the genomic editing capabilities (20, 21). This engineered variant has been successfully used in model species such as *Arabidopsis thaliana*, with promising applications for improving plant stress tolerance, including drought resistance (22, 23). GMOs with growing interest in genetically modified organisms (GMOs) and "biotech crops," regulatory frameworks are being adapted to include precision breeding techniques such as CRISPR/Cas9 (24, 25). Mutation breeding has been widely employed in various crops to enhance yield or traits related to yield, seedlessness and resistance to biotic and abiotic stress and to augment the genetic diversity of existing germplasm (26, 27).

Methodology of mutation breeding in crop improvement

Mutation breeding is a non-transgenic approach widely used to enhance yield, improve stress resistance and broaden the genetic diversity of crops. The methodology involves inducing genetic mutations using physical or chemical mutagens, followed by careful selection of desirable traits. Below is a detailed outline of the standard methodology.

Selection of plant material and choose suitable seeds, explants, or other propagules from the target crop species. Mutagen treatment physical mutagens with typically gamma rays or X-rays are used to irradiate seeds or plant tissues. The dose is optimized to balance mutation frequency and survival.

Chemical mutagens

Common agents include EMS (ethyl methanesulfonate) and sodium azide, which are applied by soaking seeds or tissues in mutagenic solutions for a specified duration. *In vitro* mutagenesis crops difficult to propagate by seed or those requiring clonal propagation (like many fruit trees), *in vitro* tissue culture techniques are used. Explants (e.g., shoot tips, callus) are treated with mutagens, allowing large populations to be handled and screened efficiently. Treated material is washed (if chemical mutagens are used) and allowed to recover and grow, either in the field or *in vitro* conditions. The first generation (M_1) is grown and subsequent generations (M_2, M_3, etc.) are screened for desired mutations. Selection focuses on traits such as yield, seedlessness, disease resistance and stress tolerance. Screening can be phenotypic (visual observation) or assisted by molecular markers for more precise identification of mutations.

Stabilization and evaluation

Selected mutants are further propagated and evaluated over multiple generations to ensure trait stability and agronomic performance. Release of new varieties mutants with stable, beneficial traits are released as new crop varieties after thorough agronomic and safety evaluation.

Mutations refer to changes in the DNA sequence or structure. Although many mutations do not result in observable phenotypic changes and are often not heritable unless selected through propagation, they differ from processes such as genetic recombination and segregation (28). Fruit crops, characterized by high levels of heterozygosity, exhibit an elevated mutation rate compared to inbred lines (29). This phenomenon can be attributed to the inherent instability of the genome in heterozygous individuals, where the disruption of genetic balance may increase susceptibility to mutational events. Consequently, mutations are frequently observed in fruit crops (27).

Types of mutagenesis: spontaneous and induced

Spontaneous mutation

Spontaneous mutations occur naturally within a population, without any deliberate human intervention. Additionally, the genes they affect are unpredictable. These mutations are caused by natural factors that can induce changes, such as electric currents, radiation, injuries, diseases, insect attacks, temperature variations, chemicals and also some of the causes of rare natural mutations (e.g., UV light, viruses, DNA replication errors) (30). Table 1 summarizes documented cases of spontaneous mutations in various fruit crops. Apple tree mutant derived from in a columnar apple mutant with larger and darker green leaves, short and strong internode, higher leaf area index, higher spur: mature branch ratio (73.5 %), per cent of short-shoots (68.8), chlorophyll A and B content (1.878 and 0.771mg g^{-1}) than standard apple.

Table 1. Spontaneous mutation influencing various traits in fruit crops

Crops	Mutant cultivars	Varieties	Year	Traits	References
Almond	Tardy nonpareil	Nonpareil	1987	Late flowering sweet kernel	(62)
Banana	Highgate	Gros Michel	1993	Semi-dwarf	(63)
	Motta poovan	Poovan	1985		
Grapefruit	Hudson	Foster	1986	Deep red flesh	(64)
Mandarin	Clausellina pongan 86-1	Owari Pongan	1987	Earliness	(14)
Mango	David Haden	Haden	1977	Larger than Haden and matures early	(65)
	Rosica	Rosado de Ica	1954	High-yielding, Early-ripening and regular bearing	(66)
Navel orange	Baianinha Navelina, Navelate, Marrs, Leng, Autumn gold, Powell summer, Winter red	Bahia Washington	2017	Lycopene accumulation, low citric acid and high sucrose	(14)
Pear	Starkrimsom	Clapps Favourite	2017	Spotting of coloured	(14)
Pomegranate	Hongmanaozi	Manaozi	2007	Deep red arils	(67)
	Hongyushizi	Yushizi	2007	Soft seeded	(68)
	Taihanghong	Mantianhong	2005	Early flowering	(67)

In fruit crops, spontaneous bud mutations, commonly referred to as bud sports, are relatively infrequent phenotypic alterations observed in the shoots of woody perennials. While these mutations are readily apparent, the underlying molecular mechanisms driving these phenotypic changes remain highly difficult (14). Among bud sports, alterations in fruit pigmentation, particularly in the anthocyanin content of red or purple fruits, are frequently observed. For instance, a bud sport of the wine grape cultivar *Vitis vinifera* "Cabernet Sauvignon", resulting in a bronze-colored mutant named "Malian" (31). Histological analysis revealed that the Malian mutant lacks anthocyanin accumulation in the subepidermal cell layers, in contrast to the parent cultivar Cabernet sauvignon, where anthocyanin is present in both the epidermis and multiple subepidermal cell layers. Similarly, a mutant of the apple cultivar "Gala", named "Grand Gala", which exhibited a 15 % increase in diameter and 38 % higher fruit weight, along with increased total soluble solids (TSS) and fewer seeds (32). Somatic mutations arising from spontaneous chromosomal rearrangements within the meristematic tissues are frequent in Indian banana cultivars. Nendran variety shows this, having given rise to several mutant derivatives are *Moongil*, *Attu nendran*, *Nana nendran*, *Nedu nendran*, *Myndoli* and *velathan* (33). Similarly, the monthan cultivar has produced numerous somatic variants, including Sambal monthan, Nalla bontha bathees, Sambrani monthan, Pidi montha and Thellatti bontha (10).

Induced mutation

Mutagens: Mutagens are agents that cause induced mutations. These agents are broadly categorized into two groups: physical mutagens, such as ionizing radiation (e.g., X-rays, gamma rays) and chemical mutagens, including alkylating agents and base analogs (27, 29). Induced mutagenesis has been widely used in plant breeding to create genetic variability and develop new fruit crop varieties with desirable traits.

Effects of physical mutation in fruit crops: Mutations can interfere with cell division and growth processes, leading to protein production, hormones and enzyme imbalances (34). Mutant varieties increased production by 34.8 million tonnes (2000-2019), with 32.7 % higher productivity than parent lines. These disruptions can also affect how plants manage water and gas exchange in leaves, potentially reducing tree height (35). Gamma radiation leads to the cell wall and cytoplasm, causing the hydrolysis of cell water and the generation of free radicals that can potentially restrict cell division (36, 37). Due to the restricted genetic diversity in the crop, researchers explored the potential for introducing variation through radiation (38). Gamma rays, with their shorter wavelength, enable greater penetration. Physical mutagens have proven particularly advantageous in certain fruit crops, inducing favorable traits such as dwarfism and early flowering. As a result, such mutations can lead to shorter plants and hinder their overall growth.

These include nuclear DNA damage is commonly considered the primary immediate outcome of exposure to IR, mitochondria and chloroplasts, as organelles housing electron transport chains and possessing their own DNA, also experience comparable adverse effects from IR observed in Fig. 1 (40, 41). Radiation energy deposition onto nucleic acid molecules generates unstable DNA radical cations, subsequently leading to their degradation. This degradation results in the breakage of phosphodiester bonds, damaging the DNA bases (42). The principal and most severe harm caused to cells due to IR exposure is the direct disturbance of the DNA chain, especially through the occurrence of single double-strand breaks (SSBs) followed by double-strand breaks (DSBs). These breaks present substantial hurdles to chromatin organization, transcription and replication, ultimately impacting essential molecular processes and jeopardizing the overall functionality of the cell (43). IR directly induces damage to bases in DNA, including oxidation (for example, the formation of guanine 8-oxo-7,8-dihydro guanine, which can mispair with adenine, resulting in G-to-T mutations), substitution, or loss of bases (44). Due to the high water content in plant cells, many IR

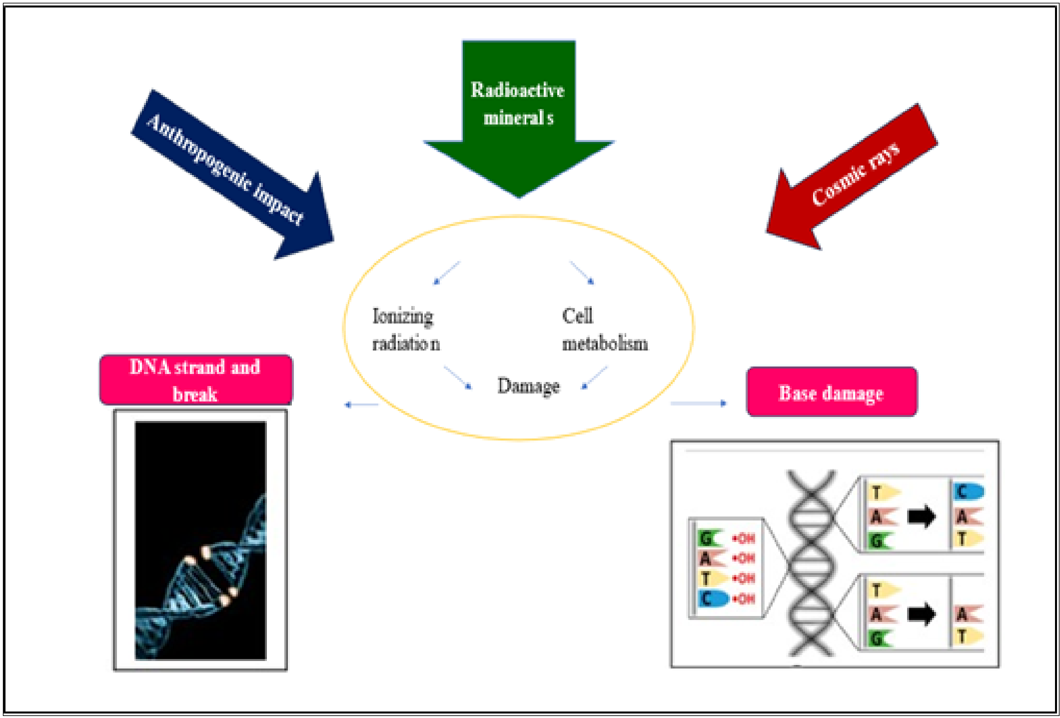

Fig. 1. Summary of ionizing radiation (IR).

effects are mediated indirectly through the production of reactive oxygen species (ROS), as depicted in Fig. 1 (45).

Ionizing radiation (IR) particularly gamma rays, can directly or indirectly damage cellular DNA. This damage can manifest as physical breaks in the DNA strand or chemical modifications to its structure. These disruptions can alter the genetic information encoded within the DNA, potentially leading to significant changes in inherited traits. While some DNA damage can be permanent, leading to cell death, most instances of DNA damage are repairable. The inherent structural properties of DNA allow for efficient repair mechanisms, such as nucleotide excision repair, to restore the original DNA sequence (46). Growth abnormalities and low germination of plants in response to higher doses of gamma irradiation are widely attributed to negative mutation, ionization of water present in cells and subsequent formation of reactive oxygen species and free radicals which can interact with other cellular molecules potentially imposing negative structural and functional changes in them (47).

Mutation induction, which primarily uses physical mutagens such as neutrons and ionizing radiation, heat treatments and X or gamma rays, has proven an efficient method in fruit breeding for enhancing commercial cultivars. Gamma rays are the most commonly used physical mutagen in breeding agricultural plant mutations (48). They are well known for causing morphogenetic and endomorphic changes in plants. Various studies have proved that plants and photosynthetic microorganisms respond to relatively low doses of ionizing radiation by exhibiting increased cell proliferation, germination rate, cell growth, enzyme activity, stress resistance and crop yields (7). Physical mutagenesis has been used to create more than 70 % of the mutant cultivars in the world (49). These beneficial effects are attributed to radiation-induced alterations in cellular structures and functions, including expansion of thylakoid membranes and enhanced photosynthetic efficiency (50). Examples of such improvements are presented in Table 2.

Effect of chemical mutation on fruit crops: Chemical mutagens, particularly ethyl methanesulfonate (EMS), sodium azide (SA) and methyl methanesulfonate (MMS), are widely used in fruit crop breeding due to their high efficiency in inducing gene-level mutations with relatively low chromosomal damage compared to physical mutagens. These agents are cost-effective, easy to handle and allow for targeted induction of genetic variability, which is crucial for improving agronomic traits in fruit crops such as banana and grapes. Chemical mutagens are alkylating substances that are highly effective in inducing mutations. Chemical mutagens have been proven to be very effective at modifying genes and their specificity of action could be determined by analyzing how different DNA bases interact with them (51). Many chemical mutagens act as inhibitors, changing the plant genome's structure. For instance, the plant cell division cycle can be stopped by colchicine and different plant species can experience changes brought on by mutagens (52).

Commonly used chemical mutagens in fruit crop improvement include DES, EMS, ethylenimine, sodium azide and colchicine. Among the various chemical mutagens used to induce mutation in fruit crops, EMS is the most effective alkylating agent that could cause mutation conversion of many characteristics, including leaf color, leaf shape, leaf number, leaf size, plant height, blooming time, flower color, flower size, etc., altered by EMS mutagenesis as detailed in Table 3 (53).

Colchicine is the most commonly and commercially used chemical for polyploidization due to its dependability and efficacy. This alkaloid, extracted from *Colchicum autumnale* L. (meadow saffron), disrupts chromosome segregation during metaphase, leading to chromosome doubling and the formation of tetraploid cells (54, 55).

Table 2. Physical mutagens role in trait improvement in fruit crops

Species	Treated cultivars	Mutagens	Dosages	Traits Improved	References
Apple (*Malus domestica*)	Amasya		29.01 Gy	Alternate bearing Increase yield and quality of fruit	(69)
	Amasya		30 Gy	Higher anthocyanin pigment Different leaf color mutants	(70)
	Oyume		7 Gy	The growth of newly extended branches was evaluated The average amount of new branch growth	(71)
Avocado (*Persea americana*)	Fuerte and Hass		30 Gy	Larger plant's oil content % increased	(72)
Banana (*Musa* spp)	Klue Hom Thong KU1 Al-beely	Gamma rays	30 Gy	Early maturity, cluster size and decreased height Resistance to *Fusarium oxyspo*rum f sp. cubense (FOC) race 4 Substantial fruit size and potential mutants showing resistance to black sigatoka disease	(73)
	Ney poovan		6.97 Gy	Increase survival percentage Increase shoot length Maximum number of shoots	(74)
	Berangan		25 Gy	Increase the number of shoots Increase root length	(75)
	Cavendish		30 Gy	Increase shoot number Increase in plant height Maximum leaf production	(76)
	Pisang ambon		10 Gy	Increase in plant height Maximum number of leaves	(77)
	Grand naine		20 Gy	Released mutant cultivar Novaria	(60)
Citrus (*Citrus* spp)	Tangor		50 Gy	Height of the fruit and its diameter Fruit yield Reduces seed number	(78)
	Mandarin cv. Nova Fino 49 Bearss		50 Gy	Increase fruit yield	(79)
	Sweet orange and sweet lemon		300 Gy	Cultivation of diploid and haploid plants	(80)
Date palm (*Phoenix dactylifera*)	Deglet Noor	Gamma rays	40 Gy	Resistance to bayoud disease	(6)
Dragon fruit (*Hylocereus undatus*)	Zi Honglong		38.5 Gy	Develop a new variety	(81)
Fig (*Ficus carica*)	Bol (Abundant)		50 Gy	Resistance to bayoud disease	(73)
Grapes (*Vitis vinifera*)	Kishmish chronic		4 Gy	Increase natural antioxidants	(82)
	Red globe Muscat ARI 516 Pembe Cekirdeksiz		15 Gy	Survival of cuttings, shoot length, leaf length and leaf width Seedless berries, Crunchy bold berries, High TSS content and high-yielding	(8, 9, 83)
Guava (*Psidium guajava*)	Gola, Surkha and Surahi		900 Gy	Enhance seedlessness	(84)
	Shweta L-49	Gamma rays	40 Gy	Maximum sprouting, short internode length Maximum number of branches and leaves	(85)
Kiwi (*Actinidia deliciosa*)	Hong yang		25 Gy	Heat stress tolerance	(86)
Lemon (*Citrus limon* L.)	Eureka 22 INTA		25 Gy	Fruit color, fruit size, seedlessness Taste and fruit setting percentage. Thornless Dwarfing habit, leaf shape, leaf length and leaf color	(87)
Mango (*Mangifera indica*)	Nekkare		35 Gy	High allelic richness	(88)
	Tommy atkins		0.25 kGy	Longer shelf-life	(89)
	Alphonso Kesar Dashehri Fazli		0.40 kGy	To reduce the severity of the pathogen and assist fungal rot Preserve firmness, skin color, Pulp color, texture and taste and extend the shelf life Reduction in physiological loss of weight and extent of ripening	(90, 91, 92)
	Arumanis		60 Gy	Percentage of graft success Increasing shoot length, bud number and total leaf number	(11)
	Zebda	Gamma rays	1.8 Gy	Increasing the shelf life of the pulp Tolerate a storage	(93)
Mangosteen (*Garcinia mangostana*)	Sabor		600 Gy	Reduce mealybug egg hatching	(94)
Oranges (*Citrus sinensis*) and Mandarins (*Citrus reticulata*)	Hongju 418, Hongju 420		10 Gy	Fruit color, size, seedlessness Taste and fruit setting percentage, thornless Dwarfing habit, leaf shape and leaf length	(27, 95)
Papaya (*Carica papaya*)	Bh65, Maradol, Shew Mee, Sunrise Solo, Tainung and V3		80 Gy	Disease resistance for black spots (*Asperisporium spp*.) Dwarfed growth	(96, 97)
Pear (*Pyrus communis*)	Fuxiang yanghongdli	Gamma rays	80 Gy	Disease resistance	(29)
Pomegranate (*Punica granatum*)	Bhagwa		84.82 Gy	Eliminating carob moth	(98)
Walnut (*Juglans regia*)	Chandler		30 Gy	High survival rates Maximum shoot length Chlorophyll density	(99)

Table 3. Chemical mutagen role in trait improvement in major fruit crops

Crops	Treated Cultivars	Mutagens	Dosages	Traits Improved	Country	References
Banana (*Musa sp.*)	Ney poovan	EMS	10 mM	Smallest shoot Long duration for rooting, the minimum number of roots and longer rooting	India	(100)
	Baxijiao		2.0 %	Cold resistance	China	(101)
	FenJiaos FJ		0.8 %	Cold tolerance and sigatoka disease resistance	China	(102)
Cape gooseberry (*P. peruviana*)	(*P. peruviana*)	Colchicine	0.1 %	Minimum plant height, larger leaf length and breadth, bigger flower size, bigger sized fruits and more fruit weight	India	(103)
Citrus (*Citrus sp.*)	Acid lime	EMS	45 mM	Increase survival rate and maximum number of leaves per plant		(104)
	Sour orange	DMS and Colchicine	0.5 %	Highest survival%, shoot length and increasing shoot numbers	India	(105)
	Sweet orange Bingtang		1.5 %	Tolerant to canker disease		(106)
Grapes (*Vitis vinifera*)	ARI 516	EMS	0.1 %	Higher performance for yield, maximum number of berries per bunch and high TSS content	India	(9)
Guava (*Psidium guajava*)	Amuyla and Punjab pink		32.50 mM	Increase germination percentage and survival of sprouts	India	(107)
Jamun (*Syzygium cumini* L. Skeels.)	Skeels	Colchicine	0.1 %	High-quality planting material	India	(108)
Mango (*Mangifera indica* L.)	Arka puneet	EMS	0.8 %	Reduced plant height, thicker stem, more branching, longer (leaf length and leaf width) and shorter (internodal length, shoot length and petiole length)	India	(13)
Mangosteen (*Garcinia mangostana*)	Masta	EMS and Colchicine	0.5 %	New branches are grown at the bottom of the plant's main stem Maximum leaf percentage	Malaysia	(109)
Papaya (*Carica papaya* L.)	Pusa dwarf, Pant papaya 1, Pusa giant and Washington	EMS	5000 ppm	Maximum TSS, sugar, fat, ash, carbohydrate, protein, carotene, minimum central cavity, maximum fruit length, fruit girth, fruit weight and pulp thickness, maximum stem girth and minimum petiole lengths	India	(97, 110)
Pineapple (*Ananas comosus* L.)	Gemilang, Bangka, Queen and Suska Kualu	Colchicine	0.05 %	Increased plant height and increased stomatal size	Indonesia	(54)
Plum (*Prunus sp.*)	Durado		0.05 %	Maximum survival percentage	Egypt	(51)
Pomegranate (*Punica granatum*)	Bhagwa	EMS	51.82 mM	High yield with better keeping quality	India	(111)
Strawberry (*F. nilgerrensis*)	*F. nilgerrensis*		0.6 %	Changes in the color, shape, number and size of leaves and the architecture of flower and plant	China	(53)

Exposure to EMS could potentially result in the impairment of GA biosynthesis. As the concentration of EMS increased, different EMS treatments on decreasing mango shoot length and stem girth became more evident in Table 3. Embryogenic callus derived from sweet orange was subjected to EMS treatment. In this situation, the increased interaction between small cell aggregates and individual cells exposed to the mutagen is intended to elevate the chances of cell mutation and simplify the isolation of mutated cells from the wild-type ones. Various pathogen toxins were employed to identify mutants exhibiting resistance to diseases, acquiring numerous mutants tolerant to diverse pathogens (56).

Achievements of *in vitro* mutagenesis in fruit crops

Random mutagenesis, employing physical and chemical agents, offers a versatile approach for inducing genetic variations across a broad spectrum of plant materials. These techniques can be applied to various plant parts, including whole plants, seeds, tubers, rhizomes, stems, buds, bulbs, pollen, leaf and stem explants, anthers, embryos, microspores, callus cultures and other plant propagules examples: Banana var. Lakatan *in vitro* shoot tips exposed to 60 Gy of gamma rays led to the development of the mutant "Novaria", which is characterized by earlier fruiting and improved agronomic traits. Banana var. Latundan shoot tips treated with 40 Gy of gamma rays produced mutants exhibiting reduced plant height and larger fruit size, traits desirable for both cultivation and marketability. Banana var. Klue Hom Thong Direct regeneration from shoot tips irradiated at 25 Gy resulted in the mutant "KU1", which displays beneficial horticultural characteristics. Pineapple var. Queen Crowns subjected to gamma irradiation generated lines with reduced leaf spines, enhancing fruit handling and consumer appeal. *In vitro* shoots exposed to gamma rays produced mutants free from russeting and with a small tree form, traits that improve pear fruit quality and orchard management (57, 58). Seeds are typically favored for mutagenesis in sexually propagated species due to their ease of handling, transportation and storage, particularly after treatment (58). *In vitro* mutagenesis, involving treating plant cells and subsequent whole-plant regeneration provides a valuable approach for inducing genetic diversity. Combining mutation breeding with tissue culture, *in vitro* mutagenesis proves more effective than conventional breeding methods, significantly improving the efficiency of mutagenic treatments are inducing variations (59). Recently, there has been enhanced efficacy in inducing mutations in plants propagated vegetatively. This approach reduces the risk of obtaining chimeric plants and increases the mutated cells expressing the mutation in phenotype. Previous studies have demonstrated successful *in vitro* shoot regeneration from pear leaf explants and further investigated the use of chemical mutagens to induce genetic variation in these regenerated plants, as illustrated in Fig. 2 (60).

Mutagenesis mediated by CRISPR-Cas9

RNA-guided tools for controlling genomic transcription, such as CRISPR interference (CRISPRi) and CRISPR-mediated gene activation (CRISPRa), are highly effective technologies for investigating how genes function (23). CRISPR Cas9 is an advanced genome editing technology that enables breeders to accurately modify genes by deleting, adding, or altering specific sections of the DNA sequence.

DNA sequencing confirmed targeted mutations in the *VvPDS* gene of regenerated grape (*Vitis vinifera*) plants, with a higher frequency of mutated cells observed in mature, lower leaves compared to young, emerging ones (61). This distribution suggests that either DNA double-strand breaks accumulate more in older tissues or that the repair efficiency in mature leaves is reduced, leading to a greater persistence of mutations in these tissues. Researchers analyzed the targeted gene regions in various transgenic grape lines and observed that mutation rates varied depending on the specific target sequence and the guide RNA used. Among the different types of mutations detected, insertion mutations were the most frequent, occurring more often than deletions or substitutions. This pattern highlights the tendency of CRISPR/Cas9-induced double-strand breaks to be repaired by non-homologous end joining, a pathway prone to insertions. In pear (*Pyrus bretschneideri*), application of CRISPR/Cas9 technology to create dwarf trees resulted in increased yield, as documented in Table 4, demonstrating the practical value of targeted genome editing for fruit crop improvement. For papaya, researchers focused on the *Ppal15kDa* gene in the *P. palmivora* pathogen, which becomes highly active during infection. By generating six CRISPR/Cas9-induced mutants of *Ppal15kDa*, they found that all homozygous mutants completely lost pathogenicity, while heterozygous mutants exhibited varying degrees of infection. This finding underscores the critical role of the *Ppal15kDa* gene in the normal progression of *P. palmivora* infection, providing valuable insights for developing disease-resistant papaya varieties. Overall, these studies illustrate the precision and versatility of CRISPR/Cas9-mediated genome editing in fruit crops and their pathogens, enabling both the functional analysis of key genes and the development of improved plant varieties with desirable traits (61).

Conclusion

Mutation breeding, especially using gamma irradiation, remains a valuable technique for crop improvement by efficiently inducing genetic variation and enhancing traits such as fruit quality, seedlessness, early ripening and stress tolerance. Gamma rays influence the plant genomic architecture, often resulting in improved germination and growth by modifying genes that control key agronomic traits. This technique has been instrumental in developing superior fruit cultivars, particularly in crops like bananas and pineapples. In parallel, CRISPR/Cas9-mediated genome editing has revolutionized plant breeding by enabling precise modifications as deletions, insertions, or targeted alterations at specific DNA sites. This approach allows breeders to directly target and improve genes responsible for desirable traits, making it an invaluable tool for modern agriculture. gamma irradiation and CRISPR/Cas9 are powerful mutagenic tools for fruit crop improvement. Gamma irradiation is best suited for inducing broad genetic variability and has a strong track record in developing new cultivars with complex trait changes. CRISPR/Cas9, on the other hand, excels in precise, targeted gene modifications, making it ideal for improving specific traits with minimal unintended effects. The choice of mutagen

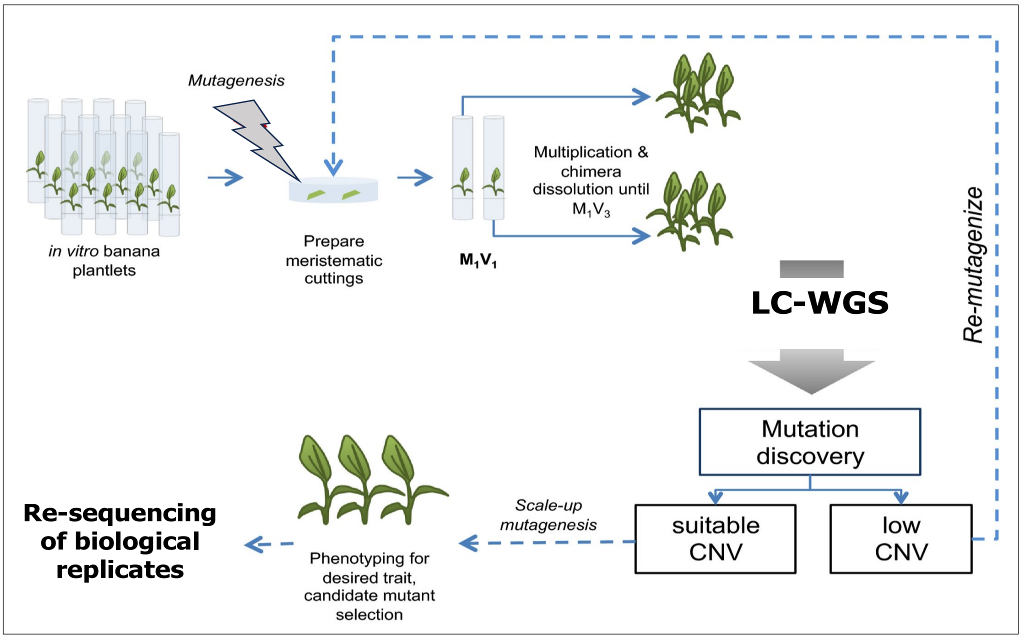

Fig. 2. A schematic diagram of *in vitro* mutagenesis. Copy Number Variations (CNV), Low-Coverage Whole Genome Sequencing (LC WGS), M_1V_1 Mutation1 Vegetative cycle1 and M_1V_3 Mutation1 Vegetative cycle3.

Table 4. Studies on CRISPR/Cas gene-editing technology applied in fruit crops

Crops	Cultivars	Target Genes	Traits Modified	CRISPR/Cas Systems	Country	References
Apple (*Malus domestica*)	Oregon spur II	*MdF3H* and *MdMYB66*	Increased anthocyanin content by might elucidate the red color phenotype.	CRISPR/Cas9	China, Germany	(112)
	Royal gala and M26	*MdMADS15* and *MdMADS221*	A significant proportion of gene modifications were achieved successfully.			(113)
	Red delicious	*THN*	Using RNA interference to hinder transcription and trigger a light-brown colony phenotype. The first successful utilization of the CRISPR-Cas9 gene editing system in the context of the apple scab fungus.			(114)
	Golden delicious and Gala	*MdCNGC2*	*B. dothidea* resistance.			(115)
		MdDIPM4	Apple susceptibility protein *MdDIPM4* is responsible for the development of the disease resistance.			(116)
	Gala	*ALS* and *PDS*	Exhibit resistance to chlorsulfuron and lines displaying albino characteristics.			(117)
		MdPDS and *MdTFL1*	Albino phenotype in 85 % of *MdPDS* lines.			(118)
	Royal gala	*CNGC2*	*B. dothidea* resistance			(119)
		IdnDH	Biosynthesis of tartaric acid.			
		DIPM-1	Increased resistance to fire blight.			(120)
		DIPM-2				
		DIPM-4				
		TFL1	Early flowering.			
		MdPDS	32 % of the regenerated plants exhibit both complete and partial albino phenotypes.			(121)
Banana (*Musa spp*)	Rasthali	*CCD4*	Regulates carotenoid catabolism and specific tissue and cultivar.	CRISPR-Cas9	India	
	Diploid banana, *Musa balbisiana*	*DMR6*	The gene provided increased resistance against banana *X. wilt* (BXW) disease and moko disease.			(122)
	Gros michel	*MaGA20ox2*	knockdown led to the formation of a semi-dwarf.			(123)
	Cavendish	*PDS*	Transforming banana protoplasts using PEG is a fast and effective way to perform temporary expression tests. Confirm the effectiveness of *sgRNA* in bananas.	CRISPR/Cas9 and CRISPR/ Cas12a	China, India	(124)
	Gonja Manjaya	*PDS*	eBSV resistance.			(125)
	Rasthali	*PDS1, PDS2*	*PDS* resulted in albinism and dwarfing. Editing efficiency dependent on Cas9 abundance. CRISPR-Cas9 modification with polycistronic.	CRISPR-Cas9	India	(126)
	Cavendish	*MaPDS*	Carotenoid biosynthesis.			(127)
	Berkeley	*gusA*	Adventitious organogenesis exists and eliminates the chimerism rate.	CRISPR-Cas9 and CRISPR-Cas12a		(128)
Berry (*Vaccinium spp.*)	*V. corymbosum* L	*PDS*	Commercially highbush cultivar to regenerate adventitious shoots.	CRISPR/Cas9	United States	(129)
	(*Vaccinium spp.*)	*CENTRORADIALIS (CEN)*	Increase the mutation frequency.			(130)
	(*V. corymbosum* and hybrids)	*GEBVs*	Highbush blueberry and superior fruit quality.			(131)
Cacao (*Theobroma cacao*)	Criollo	*TcNPR3*	Resistance to *Phytophthora tropicalis.*			(132)
Citrus (*Citrus spp.*)	Duncan grapefruit	*LOB1* promoters: *TI LOBP* and *TII LOBP*	LOB, is a S gene for citrus canker disease induced by effector *PthA4*.	sgRNAs	China, US and Europe	(53)
	Pummelo	*CmLOB1*	The S gene was identified by the citrus canker pathogen *X. citri subsp.* citri (Xcc) effector.	CRISPR-Cas9		(133)
		CsLOB1 *CsPDS*	There is a 44.4 % rate of biallelic mutations and an 11.1 % rate of homozygous mutations.			(134)
	Mini-Citrus	*FhPDS* *FhCCD4b*	Predominantly, mutations in the target genes consisted of 1-base pair insertions or minor deletions.			(135)
	Sweet orange	*PDS*	Albino phenotype.	CRISPR-Cas12		(136)
		PDS	Canker resistance.	CRISPR-Cas9		(137)
	Grapefruit	*CsPDS* *CsLOB*	Modifying these lines may enhance resistance against citrus canker disease.	CRISPR-Cas12a (Cpf1)		(137)
		LOB1	Albino phenotype.	CRISPR-Cas9		(138)

Fig (*Ficus carica* L.)	Kadota	*FcNCED2*	Mutations in target region 1 were three times more frequent than in target region 2 in both treatments.		Japan	(139)
Grapes (*Vitis vinifera*)	Pinot noir	*TMT1* and *TMT2*	Reduced sugar levels, indicating their role in sugar accumulation in grapes.		Australia	(140)
		VvbZIP36	Cas9 construct in grapevines did not result in large number of off-target mutations.			(141)
	Thompson seedless	*VvMLO3* and *VvMLO4*	Four *VvMLO3*-edited lines displayed increased resistance to powdery mildew. The mutation rate varied from 0 to 38.5 %.			(142)
		VvPR4b	Knockout lines exhibited susceptibility to *P. viticola.* It coincided with a decrease in the generation of reactive oxygen species.	CRISPR-Cas9		(143)
	Thompson seedless	*VvWRKY52*	Knockout increased resistance to *B. cinerea.*		China	(144)
	Shine muscat	*VvPDS*	Albino phenotype.			(61)
		MLO-7	Mutation efficiency of 0.1 % and 0.5-7 % were observed for targeted.			(120)
	Chardonnay	*VvPDS*	Editing occurred when the GC content reached 65 % in two different varieties. The 41B genotype demonstrating greater efficiency compared to the chardonnay genotype, even when the GC content of the *sgRNA* was the same.			(145)
Groundcherry (*P. pruinosa*)	*P. pruinosa*	*ClV1*	Increase fruit size.			(146)
Papaya (Carica papaya)	Sunrise	*Ppal15kDa*	*P. palmivora resistance*.	CRISPR–Cas9	United States	(147)
		PpalEPIC8	Cysteine protease, *P. palmivora resistance*.			
Pear (*Pyrus communis*)	*Pyrus sp.*	*PyMYB169 or PyNSC*	No lignin biosynthesis.	CRISPR/Cas12a and Cas12		(148)
		PDS and ALS	Chlorsulfuron-resistant and albino lines have been successfully generated in pear.	CRISPR-Cas9 C-to-TBE	China	(117)
		PcTFL1	Early flowering.			(118)
	Duli	*PbPAT14*	Dwarf and yellowing.			(149)
Kiwifruit (*Actinidia chinensis*)	*A. chinensis* Planch. var. chinensis	*AcBFT2*	Early-flowering.			(150)
		SyGI	Produce female flowers.	CRISPR-Cas9	New Zealand	(151)
		AcPDS	The mutagenesis rate of the PTG cassette was ten times greater than that of *sgRNAs* expressed individually.			(144)
Pomegranate (*Punica granatum*)	Wild-type	*PgUGT84A23* and *PgUGT84A24*	Special collection of gallic acid.	CRISPR-Cas9/ two sgRNAs	China	(152)
Strawberry (*Fragaria ananassa*)	Beni hope	*Fvb7-1, Fvb7-2, Fvb7-3* and *Fvb7-4*	Shoot regeneration medium, successfully inhibits tissue browning and cell death.			(153)
	Florida Brilliance	*FaPDS*	Enhanced ability to regenerate shoots.			(154)
	Chandler	*FaPG1*	Slower rate of softening, less water loss due to transpiration and showed higher resistance to damage from the gray mold pathogen.			(155)
	Benihoppe	*FvMAPK3* and *FvMKK4*	Strawberry cultivars exhibit strong resistance to low temperatures and favorable fruit quality.	CRISPR/Cas9	China	(156)
	Woodland strawberry	*FveRGA1*	Stamen and runner formation and acts sequentially with GA from bud initiation to runner outgrowth.			(157)
	Yellow Wonder	*FveMYB10*	Early developmental stages of fruit and fruit color.			(158)
	Ningyu	*TAR* and *YUCCA*	Auxin biosynthesis site for fruit set.			(159)
Walnut (*Juglans regia*)	Payne	*JrPDS*	Mutations in the target genes predominantly involved 1-base pair insertions or minor deletions.		United States	(135)

depends on breeding objectives: use gamma irradiation for broad trait enhancement and CRISPR/Cas9 for targeted genetic improvements, ensuring maximum efficiency and impact in modern plant breeding.

Future scope

As technology advances and scientific understanding grows, mutation breeding emerges as a key driver in shaping the future of agriculture. Its crucial role is foreseen in the development of crops characterized by enhanced resilience, nutritional quality and environmental sustainability.

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Authors' contributions

RVS has done writing of original draft and conceptualization. RJ, SS and GM performed revision of the draft, SG, AT, PT and SC inclusion of tables and figures, proofreading. TM and RC participated in revision, formatting and supervision. All the authors read and approved the final version of the manuscript.

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