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Mutation Breeding in Fruit Crops: Harnessing Genetic Diversity for Enhanced Varietal Traits

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Abstract

The mutation is characterized by rapid heritable changes in DNA, as opposed to genetic segregation or recombination, and occurs at a very low frequency, around one in 100,000. In fruit crops, spontaneous bud mutations, known as bud sports, are widespread, particularly in citrus, mango, and grapes, sparking interest in induced mutant breeding. Mutations, which introduce variety, drive the evolution of new forms, varieties, or species, potentially resulting in chromosomal deletions, inversions, translocations, and nucleotide base substitutions. Mutations are artificially induced by physical and chemical mutagens such as Gamma Rays, X-Rays, and EMS (Ethyl Methane Sulphonate). Conventional breeding has limits in developing fruit harvests due to their perennial nature, protracted juvenile period, and heterozygosity. Induced mutation breeding becomes an effective approach for overcoming these obstacles and increasing genetic diversity. Traditional techniques encounter difficulties such as prolonged heterozygosity, abundant fruit, incompatibility, drop, polyploidy, apomixis, and juvenile phase. Using genetic variety, whether natural or manufactured, is critical for progressing fruit tree genetics. Mutational breeding is effective for generating horticultural varieties by adding desirable features using physical and chemical mutagens. Successful results include alterations in blooming time, fruit ripening, colour changes, dwarfism induction, self-compatibility, seed lessness, self-thinning, and disease resistance. Mutation breeding produces benefits such as enhanced variety, ploidy level induction in fertility restoration and adapted species, sterile hybrids, better taste and aroma, and greater fruit size.

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Introduction

Mutations are sudden heritable changes in an organism's genetic material and subsequent traits, distinct from genetic segregation or recombination, as defined by Van Harten (1998). Further De Vries emphasized the term "sudden" to distinguish these changes from more suitable alterations observed during normal recombination processes (Roll-Hansen, 2022). Therefore, the term "mutation breeding" was coined by De Vries to specifically denote the deliberate induction and development of mutant lines for enhancing crops. The term is used more broadly to encompass the exploitation of both natural and spontaneous (bud sports, etc) mutants, besides the development of any variety possessing a known mutation from any source. Spontaneously arising mutations are rare and occur randomly in terms of time and affected genes. Enhancing fruit crop resilience amidst climate change via radiation-induced mutations is explored and to improving fruit crops through radiation-induced mutations (Maanet *al.*, 2023) resulting in deleterious effects making the organism less adapted to its environment, and in extreme cases, lethal. However, mutagenesis, with the help of physical, chemical, and biological means, is a more desirable method to induce mutations. The lack of suitable mutational breeding knowledge for fruit crops results in a strong dependence on clonally propagated cultivars, in fruit-based industries thereby resulting in a strong reluctance to change & adopt of new fruit cultivars (Janicket *al.*, 2018). Since the induced mutations can selectively alter one or a few specific traits of an elite cultivar, significantly contribute to fruit improvement without disrupting the requirements of the fruit-based industry & consumer expectations (Gurleret *al.*, 2020; Saloniaet *al.*, 2020). In fruit crops, mutagenesis has already been successfully employed to introduce many useful traits, including those affecting self-thinning, plant size, self-compatibility, blooming time, color, fruit ripening, and resistance to pathogens *etc.* (Solhjo, 2021). However, still, our knowledge related to mutational breeding in fruit crops remains rudimentary. Therefore, an attempt has been made to review the techniques & application of mutational breeding for fruit crops.

1. Mutation Breeding

In plant breeding, significant advancements have occurred through the progress of genetic engineering and molecular biology, alongside the incorporation of mutation breeding techniques. This integrated methodology has brought about a profound transformation in enhancing various aspects of fruit plants. Traditionally, plant breeding heavily relied on mutagenesis. However, the fusion of induced mutagenesis with contemporary breeding methods has expedited enhancements in both the quantitative and qualitative traits of crops (Amiteye, 2021).

The widespread impact of mutation breeding on agriculture underscores its adaptability and practicality, particularly in the context of fruit crops. Through the induction of mutations, breeders can effectively diversify plant characteristics, resulting in amplified yield, refined varietal traits, heightened resistance to diseases, and increased resilience to environmental stresses, both biological and environmental (Singha and Singha, 2024). Additionally, mutation breeding assumes a critical role

in addressing challenges brought about by climate change, facilitating faster adaptation and the introduction of novel genetic traits in fruit crops (Yali and Mitiku, 2022).

The rapid advancement in plant molecular genetics and genomics has breathed new life into mutation breeding, positioning it as a pivotal strategy in crop enhancement (Fig.1). Forecasts indicate that mutation breeding stands to benefit directly from ongoing progress in these fields, empowering breeders to fully exploit the genetic variability for trait improvement (Jegadeesan and Punniyamoorthy, 2023). Nonetheless, the efficacy of plant breeding fundamentally hinges on the accessibility of diverse genetic reservoirs tailored to specific traits.

In essence, the synergy between genetic engineering, molecular biology, and mutation breeding marks the dawn of a new era in crop improvement, equipping breeders with unprecedented tools to confront global food security challenges and bolster agricultural sustainability. (Wenefrida *et al.*, 2013).

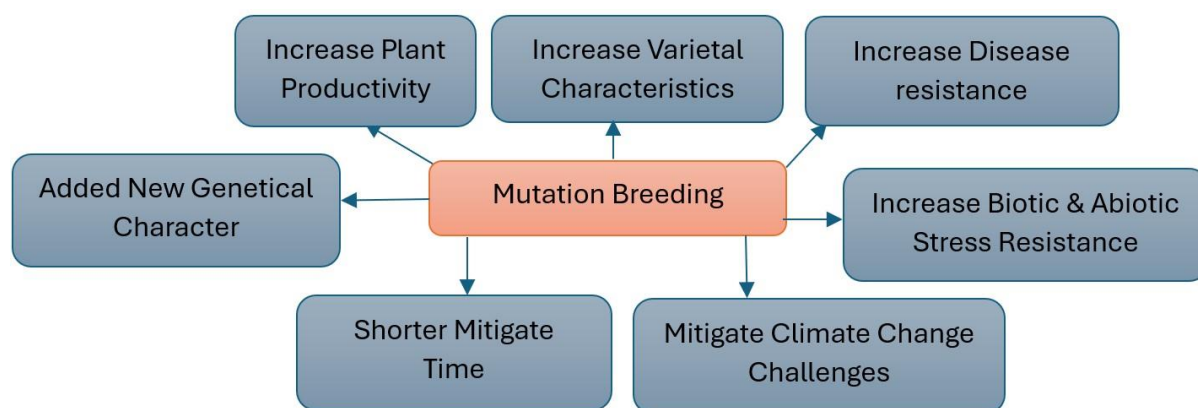


Fig.1 Mutation Breeding

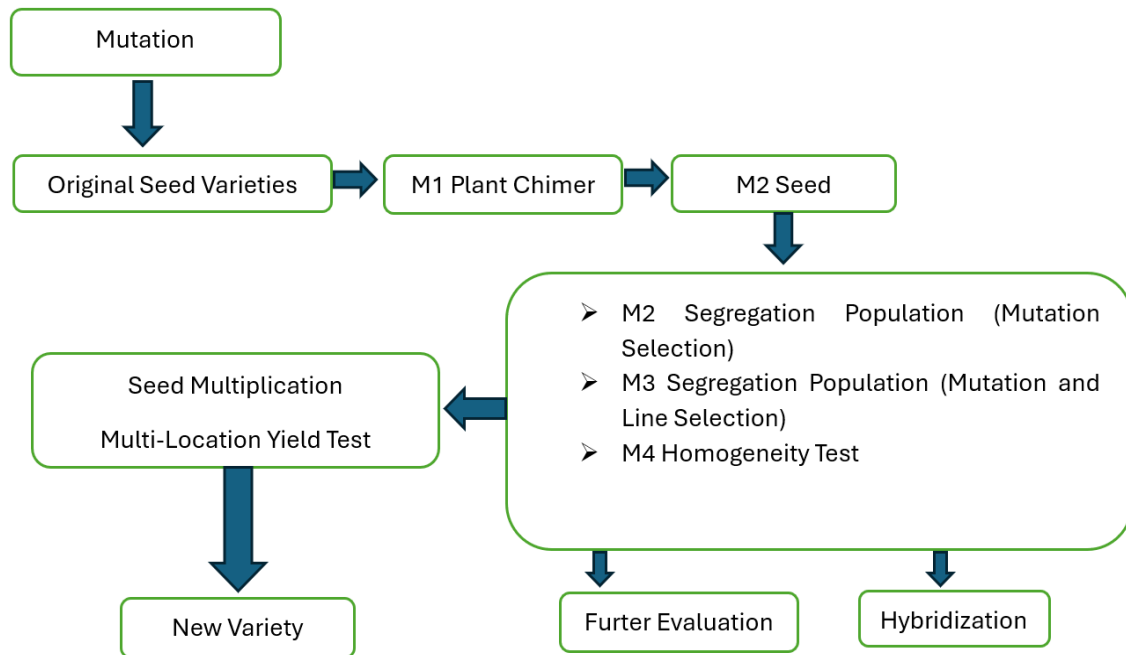
2. Method for Mutation Breeding

Every approach to mutant breeding follows a systematic series of steps. It begins with mutation induction, where plant propagules are exposed to physical, chemical, or biological mutagens (Suprasanna *et al.*, 2015). In the subsequent stage, known as mutant screening, desired individuals are chosen from a large pool of treated mutants. Seeds directly exposed to mutagens during the mutation breeding process are categorized as the M_0 generation, which gives rise to M_1 plants upon germination. The M_2 generation is produced through the self-fertilization of M_1 generation crops. Preventing cross-pollination among the M_1 population is crucial to avoid introducing new variations that may be challenging to distinguish from the effects of mutation. M_1 mutant plants are genetically heterozygous because a single mutation affects only one allele. Dominant mutations are identifiable in M_1 , while recessive mutation expression remains undetectable. Screening and selection activities begin in the M_2 generation.

The process of determining whether a selected individual is a genuine mutant, or a false one is termed mutant confirmation or verification. This process can be aided by reassessing putative mutations

under replicated and extensive conditions with a larger sample size (Fishman *et al.*, 2002). Mutant variants are screened, and desired mutants are selected based on their phenotypic traits. Phenotypic selection is more labour-intensive and specialized compared to genotypic selection (Udage, 2021). Seed multiplication for extensive field experiments follows when a mutant line exhibits promising characteristics. In this scenario, the mutant line, the mother cultivar, and other varieties are all subjected to evaluation. Field trials aim to determine whether the mutant has the potential to become a commercially viable variety that surpasses the mother cultivars.

Fig.2 Basic method involved in mutation breeding



3. Types of Mutation

3.1 Spontaneous Mutation

Spontaneously occurring mutations are almost rare and unpredictable events, both in terms of their timing and their effect on specific genes of action, giving rise to diverse forms having both significant or minor effects on the phenotype across different traits (Pascual *et al.*, 2023). These mutations can give rise to a range of forms, impacting phenotypic traits to differing degrees. Agricultural plants naturally undergo spontaneous mutations at rates ranging from 10^{-5} to 10^{-8} , which contribute to adaptability and evolutionary processes. However, the natural rate of mutation is insufficient to drive significant genetic variation in species towards desired traits (Zakir, 2018). Mutants serve an important role in modifying genetic diversity and conditions in the environment, in addition to harmful or neutral mutations seen in nature (Jiang *et al.*, 2018). Spontaneous mutations are exceedingly unusual and unpredictable occurrences, both in terms of timing and gene location. Mutations can be damaging or even deadly to an organism, but they can also produce helpful recombinant genotypes in future generations. Natural mutations and bud sport are both terms used to describe spontaneous mutations. Mutations can impact natural populations, making them beneficial

for breeding purposes. Although naturally advantageous mutants like Davis Haden and Rosica have been identified, there is Michel, also known as Big Mike, is a banana variety distinguishable from those in the Cavendish subgroup, characterized by traits like bottle-necked fruit, ripening to a full yellow colour, green or pale pink and bright red under sheath, short pedicels, and extreme susceptibility to Panama disease (Mandal *et.al.*, 2023).

A new plant component develops as a sport and exhibits morphological distinctions from the rest of the plant. The new character might be shaped or colored like leaves, a flower, or a branch. New sports with desired characteristics are vegetatively propagated to create a new fruit cultivar (Datta, 2023). Horticulturists propagate these bud sports because they retain other desirable traits of the parent plant. The clonal or vegetative propagation method is considered vital for propagation to produce commercial products generally in horticultural crops (Foster and Aranzana, 2018; Timbadiya *et al.*, 2023)).

The bud mutations serve as an important source of variability, leading to variations with desirable traits such as superior fruit quality (Atayet *et al.*, 2018). Bud sport mutations at the plastid, genic, chromosomal, or genomic levels originate in a shoot apical meristem and spread through mitosis to the whole bud and subsequent buds (Prudencio *et al.*, 2022). Unstable genotypes are limited to specific branches of the tree and can only be spread by clonal procedures.

Spontaneous mutations appear more often in specific sections of the genome (Jarniet *et al.*, 2014). Clonal or vegetative propagation is essential in producing commercial goods, particularly in horticultural crops (Agrawalet *et al.*, 2023). This technique guarantees that offspring maintain the characteristics of the parent plant with minimal variations since they stem from a single source. Clonal propagation offers significant advantages, such as enhanced adaptability to diverse environments due to genetic heterogeneity. However, as clonal reproduction becomes more widespread, there is a decrease in genetic diversity (Aysanovet *et al.*, 2019). The clonal or vegetative propagation method is crucial for the production of commercial horticultural crops. Several mutant cultivars have been reported in several fruit crops such as mango (Nayak *et al.*, 2023), banana (Auxilia and Shabha, 2017), grapefruit (Rana *et al.*, 2020), Pear (Thakur *et al.*, 2023), Mandarin (Lamoet *et al.*, 2017), Navel Orange (Hazarika, 2023). Identified (Table 1).

Table 1. Spontaneous Mutation in Different Fruit Crop

Crop	Mutant Cultivar	Variety	Nature of Mutation and Traits
Mango	Rosica Haden	Rosado de Lea Davis Haven	regular bearing, larger fruit size and Bud sports Precocious.
Banana	Gros Michel Poovan	Highgate Motta Poovan	semi dwarf Sports, Sports,
Grapefruit	Hudson	Foster	deep red flesh, Bud sports,
Pear	Starkrimson	Clapp's Favourite	Spotting of coloured, Bud sports.
Mandarin	Clausellina Pongan 86-1	Owari Pongan	Bud sport

Navel Orange	Winter Red, Anutuma Gold, Baianinha, Naveline, Powell Summer, Navelate, Marrs, Leng.	Bahia Washington	Limb sports
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Lamoet *et al.*, (2017) and Maurya *et al.*, (2022)

3.2 Induced Mutation

Genetic variation is the mainstay that plant breeders require to produce new and improved cultivars. The opportunity to obtain novel traits exists through the induction of mutations. Induction of mutations is an effective method to enhance natural genetic resources (Wang *et al.*, 2016). Induced mutations have played a significant role in meeting challenges related to world food and nutritional security by way of mutant germplasm enhancement and their utilization for the development of new mutant varieties (Raina *et al.*, 2016). Such varieties can be developed with the help of artificial or induced mutations that can occur in the presence of different mutagenic agents, viz., chemical (alkylating antineoplastic agents, hydroxylamine, azide *etc.*), physical (X-rays, gamma rays, beta particles, ultraviolet rays, alpha particles *etc.*) and biological (bacterial and Virus) (Prudencio *et al.*, 2016) agents that can cause and enhance the frequency of mutations (Singh *et al.*, 2021). Chemical and physical mutations are claimed to be more useful in plant breeding programs as compared to other mutations, especially spontaneous ones, which are rare events and may not withstand for a longer duration (Ulukapi and Nasircilar, 2018). Chemical mutagens are considered highly effective in producing optimal mutagenesis however, they are highly toxic and are not recommended for tissue culture plants. Whereas, physical mutagens have widely been used to induce hereditary abnormalities, and more than 70% of mutant varieties have already been developed using physical mutagenesis (Roychowdhury *et al.*, 2013; Kiran *et al.*, 2022). Induced mutations can play a pivotal role in enhancing not only the key agronomic properties but yield & yield attributing characteristics in different fruit crops. Notable improvements include disease resistance in Japanese pear and peach, reduced height in pomegranate and papaya, compactness in Sweet Cherry, seed lessness in Citrus, and Guava and earliness in Apples *etc.* (Sattar *et al.*, 2021; Ragini, *et al.*, 2019) with help of different mutation causing agent known as mutagens.

4. Mutagen

A mutagen is a substance, either occurring naturally or synthesized, capable of triggering genetic mutations in plants. These mutagens are typically divided into two categories: physical, chemical, and biological mutagens, which encompass ionizing radiation and various chemical agents. Both types are employed to induce mutations, with gamma rays and ethyl-methane sulphonate (EMS) being particularly common for this purpose (Kamatyanatt *et al.*, 2021).

4.1 Physical Mutagens

In the past 80 years, physical mutagens have been used widely for inducing hereditary aberrations and more than 70 percent of mutant varieties were developed using physical mutagenesis (Mbaet *al.*, 2012). beta particles, gamma rays, fast neutrons, X-rays, slow neutrons, and UV rays are the six most employed physical mutagens to cause plant mutations (Udage,2021). Physical mutagens are divided into two types: ionizing radiations (e.g. gamma rays, beta particles, alpha, neutrons, and X-rays) and non-ionizing radiations (UV rays)(Khursheed, 2021).

Radiation refers to energy that moves as particles or waves (Ishimaru, 2017). These electromagnetic (EM) spectra have high energy levels and can shift electrons from atoms' nuclear orbitals. Ionizing radiation is named after the fact that it causes atoms to form ions (Baranov,2017). X-rays were initially employed to induce mutations, but gamma rays from radioactive cobalt (^{60}Co) are now often utilized. Radiation-induced mutation is the most common approach for creating direct mutant varieties, accounting for around 90 percent (64% with gamma-rays and 22% with X-rays) of the varieties developed to date using mutational breeding(Jain,2005). This ionizing radiation can penetrate deeper into plant tissue and can cause various chemical alterations resulting in different gene mutations (Ainsbury *et al.*, 2018). Gamma radiation has produced several beneficial mutants and remains a promising method for enhancing vegetatively propagated plants (Kashtwari *et al.*, 2022). Recently it has been reported that DPM25, a mutant of banana (*Musa* spp., AAA group, Cavendish subgroup), had better output and fruit size, besides being resistant to Fusarium wilt, a potentially catastrophic disease (Dale *et al.*, 2017). Similarly, Gamma irradiation was used to select putative-resistant mutants for black Sigatoka disease from the susceptible banana variety 'Grande Naine' (Penna *et al.*, 2021; Rani *et al.*, 2023). Furthermore, it has been reported that the different plant parts have distinctly been subjected to different types of treatments *viz.* gamma ray treatment for shoot tips, shoots, leaves, and shoot clumps, while X-rays were applied to shoot tips and shoots. Hence, extensive use has been made of gamma rays and X-rays as ionizing radiation for fruit tree improvement (Bisht, *et al.*, 2021).

Moreover, physical mutagens are much more stable and cost-effective, but they can result in multisite mutations of varying magnitude, potentially impacting the non-target genes. The 10-year research revealed late flowering, dwarf growth, pest, and disease resistance (Prudencio *et al.*, 2022; Naikodi *et al.*, 2021). Several research investigated irradiating sweet cherry buds (dormant scions) to optimize dose and rescue beneficial mutations Yang and Schmidt successfully cloned a mutant type of X-ray-irradiated cherry leaves.

Similarly, ionizing radiation, such as gamma irradiation has been favoured for generating genetically stable and disease-free mutants due to its shorter wavelength as compared to X-rays and neutrons, enabling deeper tissue penetration (Pogue *et al.*, 2021). Whereas thermal neutrons are used on pollen (Khursheed *et al.*, 2021) this may be due to the action of ionizing radiations resulting in the development of DNA strands, thereby, enabling cross-linking of chromosomal breakages, nucleotide substitution, and nucleotide deletion (Venset *et al.*, 2018) under the presence of different external factors

affecting irradiation such as oxygen and moisture contents, temperature, and post-irradiation storage conditions (Khan and Abraham, 2010).

Table 2. Physical Mutagens Used for Inducing Mutation

Fruits	Mutagen	Sources	Plant Material	Lethal Dosages	Reference
Banana	Gamma Rays	Radioisotopes and Nuclear Reaction	Shoot Tip	30-35 (Triploid)	Lamoetal., (2017)Hasim <i>et al.</i> , (2021)
Japanese Plum	Gamma Rays	Radioisotopes and Nuclear Reaction	Shoots	30	Predieri and Gatti (2000); Lamo <i>et al.</i> , (2017)
Pear	X- Rays	X- Rays Machine	Shoot Tip	11	Rui Liu (2022); Maurya <i>et al.</i> ,(2022)
Apricot	Thermal Neutrons	Nuclear Reactor	Pollen	>75- <100	Wu <i>et al.</i> , (2011); Maurya <i>et al.</i> ,(2022); El-Sabagh, A. S., Barakat, M. N., &Genaidy, E. E. (2011)
Apple	Gamma Rays	Radioisotopes and Nuclear Reaction	Leaves	>10- <20	Ulu Kapi, & Nasircilar,(2015) Lamoet <i>al.</i> , (2017)
Cherry	X- Rays	X-Ray Machine	Shoots	>94-<98	Maurya <i>et al.</i> ,(2022);Ishtiaqet <i>al.</i> , (2023) Person and E.S. Jackson (2013)
Strawberry	Gamma Rays	Radioisotopes and Nuclear Reaction	Shoot Clumps	>50- <100	Lamoetal., (2017) Jesus Filho <i>et al.</i> ,(2018)
Kiwi	Gamma Rays	Radioisotopes and Nuclear Reaction	Leaves	40-60	Pathirana, R. (2021). Kaur <i>et al.</i> , (2018)

4.2Chemical Mutagen

Chemical mutagens are typically perceived as having a gentler effect on plant materials (Mba *et al.*, 2010). An advantage of these agents is their application simplicity, as they don't require intricate equipment or facilities. Furthermore, the ratio of desired mutations to unwanted alterations is generally higher with chemical mutagens compared to physical mutagens (Oladosuet *al.*, 2016). However, Researchers began searching for alternative methods to induce mutations due to the increased rates of chromosome abnormalities and associated harmful effects caused by ionizing radiation. (Celik and Atak, 2017). Consequently, a diverse range of chemical mutagens has been identified. However, the wide variety of these chemical mutagens complicates the establishment of

common rules and conditions for their use. However, it's important to note that chemical mutagens are often carcinogenic, necessitating extra precautions for health protection since, during the process of mutagen application, the material is immersed in a mutagen solution to induce mutations (Ames and Gold, 2018).

It has been reported that the chemical mutagens have demonstrated remarkable efficacy in triggering authentic gene mutations, with their specificity of action being assessed through interactions with diverse DNA bases. A variety of chemical mutagens, including alkylating agents such as Ethyleneimine (EI), Diethyl sulphate (DES), Ethyl methane sulphonate (EMS), Ethyl nitroso urethane (ENU), Ethyl nitroso urea (ENH), and azides, are generally depended upon to induce mutations in various fruit crops due to their potency and ease of disposal by hydrolysis, efficient role in generating soma clonal variation, etc. (Riaz and Gul, 2015; Gadoet al., 2018). Furthermore, compared to physical mutagens, chemical mutagens are more inclined to induce gene mutations rather than chromosomal alterations (Table. 3).

Different chemical mutagens and Alkylating agents, such as MNU, ENU, MMS, EMS, DMS, DES, MNNG, ENNG, NDMA, and NDEA, are widely used for inducing mutations in plants. These agents introduce methyl or ethyl groups to bases, leading to degradation, mispairing during replication, and mutations. Azide and hydroxylamine have similar effects as alkylating agents. Antibiotics, such as mitomycin C, streptonigrin, azaserine, and actinomycin D, are associated with chromosomal abnormalities and male sterility. Nitrous acid induces deamination, leading to transitions during replication. Acridines, such as acridines orange, insert themselves between DNA bases, disrupting the DNA double helix structure and leading to frameshifts. Base analogs, such as 5-bromouracil (5-BU), 5-bromodeoxyuridine, 2-aminopurine (2AP), and maleic hydrazide substitute regular bases during replication, resulting in transitions and tautomerization. These mutagens are used in various research and industrial applications, but their potential side effects and risks must be carefully managed. Ethyl methane sulphonate (EMS), a type of alkylating agent, is one of the most used chemical mutagens for inducing mutations in plants, including bananas and grapes (Gadoet al., 2018). EMS is well-known for its efficacy and efficiency in producing somaclonal variants in crop plants. During the procedure, banana shoot tips are frequently exposed to mutagens before being regenerated (Penna et al., 2019; Rajan and Singh, 2021). The use of chemical mutagens has also been reported in bananas (*Musa spp.* AAA group) for the development of fusarium wilt-resistant varieties (Saraswathi et al., 2016).

Researchers have also utilized chemical mutagens to create banana varieties that are resistant to fusarium wilt. Despite the great findings obtained with chemical mutagens, researchers are now investigating alternate approaches, such as physical and biological mutagens, which leave no residues after treatment. This is because chemical mutagens are very reactive and must be used in fresh batches for best outcomes.

Table. 3 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)

Maurya *et al.*, (2012)

4.3 Biological Mutagen

Agrobacterium-based chromosomal integration and transposon-based chromosomal integration are two biological mutagens that are being studied intensively (Bhattacharya *et al.*, 2023). Plant breeders are primarily concerned with the points of variation that may be formed by mutation in a very short period (Harten, 1998). Typically, it takes 6 to 7 years to breed a plant and provide a more stable variety than its parent (Bradshaw, 2017). This issue may be solved by implementing mutation breeding, which takes no time at all to breed a variety or cultivar with superior characteristics (Ahmar, 2016). The fruit crop development initiative using mutant breeding began ninety years ago. Even though it was established earlier, numerous advancements are still being made in the process of

generating mutations in fruit crops (Sivasankar, 2023). Genetic engineering using genetic transformation technologies is now widely used to enhance plants (Cunningham, 2018). Transgenic breeding is significant in the enhancement of fruit crops because breeding is limited by issues such as extended life cycle, propagation technique, high heterozygosity, and reproductive obstacles (Shivran, 2022). *Agrobacterium tumefaciens* mediation is a popular transformation approach because it promotes effective tissue and cell culture, somatic embryogenesis, and plant regeneration. Fruit crop genetic transformation has been highly effective in improving disease resistance, drought, cold, and salt tolerance, improved plant development patterns, and fruit quality (Krenek, 2015). Gala and Golden's Delicious varieties were used for apple transformation, while Chardonnay, Thompson Seedless, Sugarone, and the model genotype Microvine, for grapevine. In grapevine, CRISPR Cas9-mediated mutagenesis took out transcription factor VvWRKY52 and made it resistant to *Botrytis cinerea* (Blascoet al., 2015).

In addition, 22 mutant plants were developed from 72 T-DNA-inserted plants during the pioneer production process. The critical gene responsible for citrus canker produced by *Xanthomonas citris* sp. citri (Xcc) was CsLOBI (Afiya, 2021). CsLOBI stands for Citrus sinensis Lateral Organ Boundaries. Duncan grapefruit (*Citrus paradise*) includes two CsLOBI alleles and employs CRISPR Cas9 to mutate the coding area of CsLOBI in the two alleles, making citrus canker resistant (Paterson, 2005). In Banana, comparative transcriptomics of the resistant wild-type banana *Musa balbisiana* and susceptible banana Pisang Awak knocked out single or multiple susceptibility genes (such as MLO13, DMR6), transporter genes (such as SWEET14), and negative regulators (e.g., E3 ubiquitin ligases) that provide resistance to banana *Xanthomonas wilt* (BXW) caused by *Xanthomonas campestris* sp. *musacearum*. CRISPR activation (CRISPR) technology was used to activate endogenous *Musa* defense genes such as disease resistance, pathogenesis-related genes, receptor kinases, and antibacterial proteins. Editing the eIF gene in bananas offers resistance to Banana Bunchy Top virus (BBTV) illness (Ferguson *et al.*, 2013; Babu and Dev, 2022). The 'initial phase' of transgenic fruits involved the transformation of fruit crops such as apple, pear, plum, cherry stock, grapes, walnuts, kiwifruit, citrus, and European chestnut using the *Agrobacterium* technique. In the second phase of development, RNAi technologies were primarily used to generate GM fruit crops (plum, cherry, and apple), as well as to fine-tune protocols for *Agrobacterium* genetic transformation (blueberry, sour cherry), marker-free plants (apple, citrus, and apricot), and to commercialize some transgenic events, such as non-browning apples. Phase II focused on the development of genome editing techniques for fruit crops (apple, grape, sweet orange, grapefruit, and kiwifruit). The acceptance of GM cultivars in key food crops has been amazing, with enormous areas under cultivation and economic advantage (Limeret *et al.*, 2017). According to a 2019 ISAAA research, GM crops are produced on 190.4 million hectares across 29 countries, a nearly 112-fold increase from 1.7 million hectares in 1996. This involves cultivating GM fruit crops in the United States (papaya, squash, and apple), China (papaya), and Costa Rica (pineapple). GM virus-resistant papaya is the most commonly grown genetically altered fruit, followed by virus-resistant squash, apples, and pineapple.

5. Somaclonal Variation

Somaclonal variation has resulted in the identification of numerous variants demonstrating enhanced resistance to pests, herbicides, and diseases (Anilet *et al.*, 2018). This phenomenon serves as a valuable tool for introducing diversity in fruit improvement. Noteworthy examples of soma clones exhibiting resistance include those in strawberries against *Fusarium oxysporum* f.sp.fragariae, *Alternaria alternate*, and *Phytophthora cactorum* (Krishna *et al.*, 2016). Additionally, apple rootstocks MM106 and M26 (*Malus pumila* Mill.) have produced somaclones resistant to the root-knot nematode (White and *Meloidogyne incognita* Kofoid) (Talaie *et al.*, 2004). In the case of Bintang sweet oranges, (Hongjuan *et al.*, 2015) employed *in vitro* mutagenesis with 0.5% EMS to generate somaclones tolerant to citrus canker disease. The resulting somaclones, known as DG-2, exhibited resistance to canker disease. Grapevine produced genotypes with increased tolerance to salinity (Afiya *et al.*, 2021).

6. Conclusion

Mutation is a key breeding approach for generating variety in fruit crops. This method can quickly improve features including dwarf plants, earliness, tolerance, and resistance to diseases and pests. Mutation detection and genotypic selection have revolutionized fruit crop breeding and genetics. Induced mutation can accelerate the breeding process for genetic variety or multiplication. It promotes the development of commercial cultivars to achieve nutritional security and generation of livelihoods for the growers besides, maintaining the industrial prosperity.

Table. 4 Achievement Through Mutation Breeding in Fruit Crop

Crop	Cultivars Released	Country	Year	Mutagens	Traits
Fig	Bol	Russian Federation	1979	Gamma Rays (50-70 Gy)	Non-Define
Grape	Fikreti		1986	Gamma Rays	Earliness
Indian Jujube	Mahong	Viet Nam	1986	MNH (0.02-0.04%)	Fruit Morphology
	Dao tien	Viet Nam	1986	MNH (0.02-0.04%)	–
Lemon	Eureka 22 INTA	Argentina	1987	X-Rays (10 Gy)	Fruit Set, Quality
Grapefruit	Star ruby	USA	1970	thN	Parthenocarpy
	Rio red	USA	1984	thN	Fruit Colour
Loquat	Shiro-mogi	Japan	1982	Gamma Rays (200 Gy)	Fruit Size
Mandarin	Zhongyu 7	China	1985	Gamma Rays (100 Gy)	–
	Zhongyu 8	China	1986	Gamma Rays (100 Gy)	–
	Hongju 420	China	1986	Gamma Rays (100 Gy)	–
	Nibakinnow	Pakistan	2017	Gamma Rays (20 Gy)	Sparse Seeded
	Pau kinnow- 1	India	2017	Gamma Rays (30 Gy)	Parthenocarpy
Apple	Golden haidegg	Austria	1986	Gamma Rays (50 Gy)	Fruit Size
	Mcintosh 8F-2-32	France	1970	Gamma Rays (50 Gy)	Skin Colour
	Black join BA 2 520	France	1970	Gamma Rays (50 Gy)	Fruit Colour
	Balrene	France	1970	EMS	Earliness
	Lysgolden	France	1970	Gamma Rays	Rust Resistant

				(50Gy)	
	Courtavel	France	1972	Gamma Rays (50Gy)	Shortness
	Courtagold	France	1972	Gamma Rays (50Gy)	Shortness
	Senbatsu-fuji-2-kei	Japan	1985	Gamma Rays (60Gy)	Fruit Colour
	Shamrock	Canada	1986	Gamma Rays	Earliness
Banana	Novaria	Malaysia	1993	Gamma Rays, in Vitro	Earliness
	Kluehom thong kul	Thailand	1985	Gamma Rays, in Vitro	Bunch Size
	Fuxuan 01	China	2005	Gamma Rays	–
	Al-beely	Sudan	2007	Gamma Rays	–
	Pirama 1	Indonesia	2019	Gamma Rays (30 Gy)	–
Plum	Spurdente-ferco	France	1988	Gamma Rays	Earliness
Pomegranate	Karabakha	Russian Federation	1979	Gamma Rays (50-70 Gy)	Non-Define
	Khyrda	Russian Federation	1979	Gamma Rays (50-70 Gy)	Dwarfness
Peach	Magnify 135	Argentina	1968	Gamma Rays	Fruit Size
	Plovdiv 6	Bulgaria	1981	Gamma Rays (10 Gy)	Yield
	Shaji 1	China	1985	CO2 Laser	Fruit Quality
	Fuxiangyanghongdi	China	1983	Gamma Rays (2.5 Gy)	–
Pear	Gold nijisseiki	Japan	1993	Gamma Rays	Disease Resistant
	Kotobuki shinsui	Japan	1996	Gamma Rays	Disease Resistant
Sweet Cherry	Lapins	Canada	1983	X-Rays	Larger Size, Firmer
	Stella	Canada	1968	X-Rays (50 Gy)	Self-Fertile
	Stella 16A-7	Canada	1972	X-Rays (50 Gy)	Compact Growth
	Compact stella 35b-11	Canada	1974	X-Rays (40 Gy)	Compact Growth
	Sunburst	Canada	1983	X-Rays (50 Gy)	Fruit Size
	Burlat C1	Italy	1983	Gamma Rays	Compact Growth
	Nero II C1	Italy	1983	Gamma Rays	Compact Growth
	Ferrovio spur	Italy	1992	X- Rays (4 Gy)	Shortness
	Super 6	Japan	1997	Colchicine	–
	Roman nishiki	Japan	2001	Colchicine	–
	Aldamla	Turkey	2014	Gamma Rays (25 Gy)	–
	Burak	Turkey	2014	Gamma Rays (50 Gy)	–
Sour Cherry	Podorodnayamichurina	Russian Federation	1977	X-Rays	Fruit Size
	Polukarlikorlovskoi	Russian Federation	1979	Gamma Rays	Dwarfness
	Polukarlikurgenevk	Russian Federation	1979	Gamma Rays	Dwarfness
	Karliksamorodka	Russian Federation	1979	Gamma Rays	Dwarfness
	Nishinazao (dt2008)	Japan	2009	Ion Beams	–
Orange	Xuegan 9-12-1	China	1983	Gamma Rays (100 Gy)	Parthenocarpy
	Hongju 418	China	1983	Gamma Rays (100 Gy)	Parthenocarpy
	Valencia 2 INTA	Argentina	1987	X-Rays (20 Gy)	FruitQuality
Papaya	Pusananha	India	1987	Gamma Rays (150 Gy)	Dwarfness
Almond	Supernova	Italy	1987	Gamma Rays (30 Gy)	Lateness

Apricoat	Early blenheim	Canada	1970	ThN	Earliness
Kinnow	Pau kinnow-1	India	2016	Gamma Rays (30 Gy)	Parthenocarp
Pummelo	Pamelonambangan	U.K	1700	Gamma Rays (20 Gy)	–

Mohammad,(2001); Rattanpal *et al.*,(2015); Mariana *et al.*,(2018); Maurya *et al.*, (2022)

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