Genetic Variations in Fruit Trees

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Fruit trees provide essential nutrients to humans by contributing to major agricultural outputs and economic growth globally. However, major constraints to sustainable agricultural productivity are the uncontrolled proliferation of the population, and biotic and abiotic stresses. Tree mutation breeding has been substantially improved using different physical and chemical mutagens.

mutagenesis TILLING genome editing targeted mutation CRISPR-Cas transgene-free fruit trees biotechnology

1. Introduction

Conventional breeding has been the sole source of genetic improvement in fruit crops for various traits. Classical approaches to introduce a promising trait in an elite cultivar require the introgression of related alleles through multiple generations of selection. For example, the introduction of a disease-resistance trait into a high yielding cultivar is commenced by crossing it with a disease-resistant cultivar, followed by recurrent backcrossing with the elite parent to sustain the genetic potential of the elite cultivar besides conserving the newly introduced resistance allele. Usually, the whole process encompasses several generations to stabilize the resistance allele in the elite background. Fruit crop breeding have certain limitations, which may include outcross reproduction, prolonged juvenility, and enormous genome landscapes [1][2]; therefore, it requires decades to improve such traits. The obligate outcrossing nature of fruit trees amalgamates classical breeding for genotypic and phenotypic traits. A relevant example to illustrate this dilemma is the development of resistance to apple scab. Hough et al. [3] conducted a wide range of crosses between an elite apple cultivar and a genetically compatible wild-type cultivar as the source of resistance to apple scab. However, over several decades of continuous breeding, the resultant cultivars lost the fruit quality traits [4]. The application of marker-assisted selection, such as marker-assisted breeding (MAB), marker-assisted selection (MAS) and genome-wide association mapping (GWAS) for quantitative trait loci (QTLs), may contribute to shorten the selection process, but not bypass the generations of backcrossing [5]. For example, apples, cucumbers, mandarins, peaches, and strawberries have been substantially improved [6][7][8]. Fast-track breeding approaches may possibly overcome extreme juvenility in fruit trees via the transgenic expression of the desired genes. The breeding time for fruit trees can be shortened to one-fifth of the conventional crossbreeding approaches [9]. For example, the apple cultivar ‘Pinova’ was transformed to impart early flowering by expressing a MADS-box gene from *Betula pendula* [10]. In another study, null segregants of fire blight and apple scab resistant apples were generated within seven years [11]. Similarly, Endo et al. [7] successfully substituted the genetic background of mandarin through an integrated transgenic approach and MAS to transfer CTV resistance

from a transgenic trifoliate orange. However, to obtain the null segregants, the transgene should be segregated out from the elite parental background through backcrossing with the recurrent parent. The detachability of the T-DNA transgene can be confirmed through comparative genomic hybridization (CGG) and next-generation sequencing (NGS) approaches [9]. In fast-track breeding, MAS plays a critical role to increase the selection efficiency in the backcrossed progenies.

Several new strategies were introduced in the middle of the 20th century to enrich the genetic diversity of fruit trees. Mutagenesis has been used to facilitate plant breeding since the 1920s with the discovery that mutations induced with physical (gamma irradiation) or chemical mutagen treatment can be inherited [12]. Importantly, with the discovery of X-rays, a subsequent series of induced mutations were conceptualized in plants and animals (**Figure 1**). In 1934, the first commercial mutant tobacco variety was produced [13]; since then, mutant crop cultivars have been continuously registered globally (**Figure 2**A,B). It was not until 1963 that the first mutated apple cultivar "Mori-hou-fu 3A" was developed in Japan through gamma rays. The following year, a sweet cherry (*Prunus avium* L.) cultivar "Compact Lambert" was developed in Canada (**Table 1**). The use of chemical mutagen "EMS" was successfully applied for mutating apples to develop another mutant cultivar "Belrene" in France in 1970. The application of physical mutagens was also successful in tree breeding. For example, grapefruit (*Citrus* × *paradisi*) cultivar "Rio Red" and clementia (*Citrus celementina*) cultivar "Nero" were developed using thermal neutrons and fast neutrons in 1970 and 2006 in USA and Spain, respectively. Various hybridization methods were also developed to produce hybrids between sexually incompatible species by disrupting the meiotic cell division to form polyploids, followed by the restoration of meiosis. Additionally, hybridization approaches also included chromosomal additions/subtractions or the fusion of protoplasts from sexually incompatible species [14]. The genetic background of the elite crop cultivars was further broadened through chemical or physical mutagenesis to increase the desirable alleles in the elite lines.

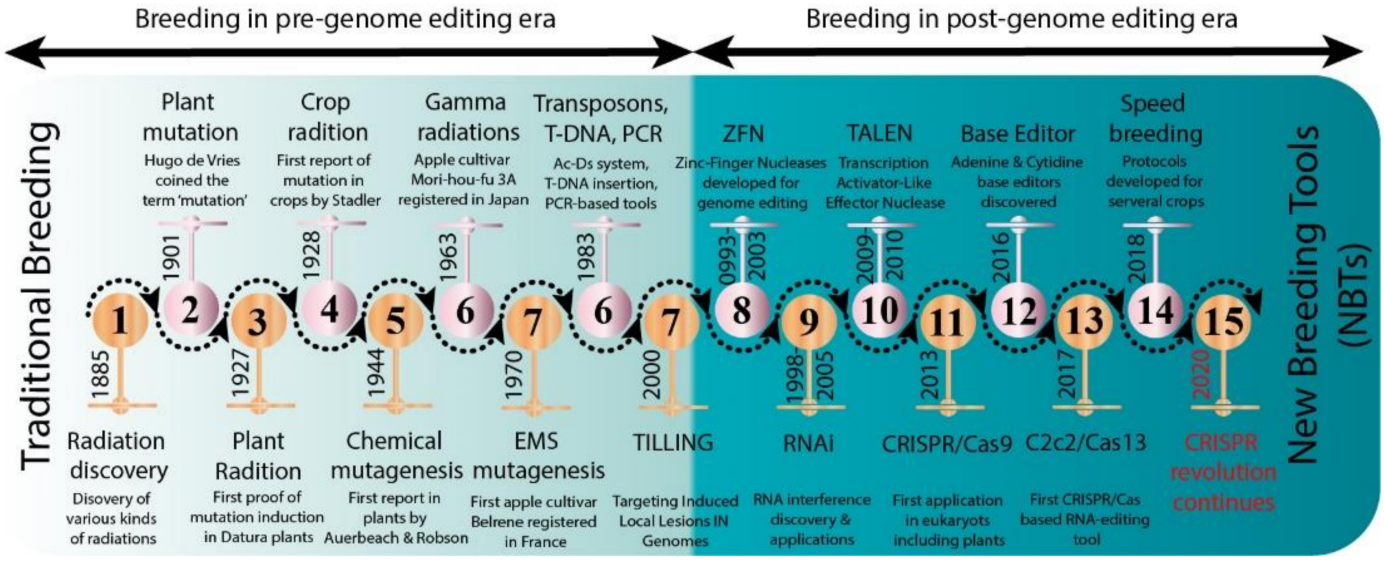

Figure 1. Historic timeline for mutagenesis in plants.

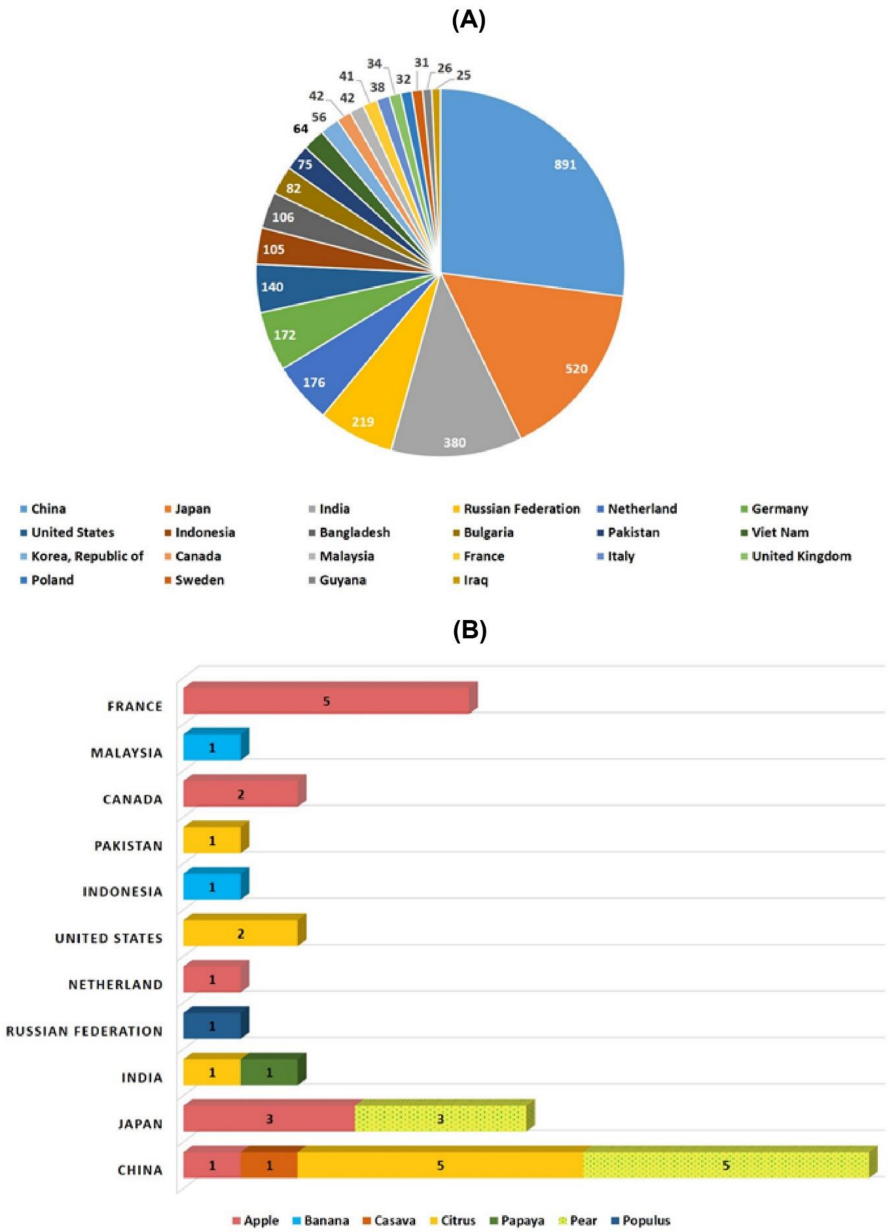

Figure 2. Number of mutant varieties released in top 22 countries (**A**) and number of mutant tree varieties of assorted fruit species in selected countries (**B**).

Table 1. Country-wise varietal approval developed by mutagen treatment.

Common Name	Botanical Name	Variety	Mutagen Source	Country	Year of Registration
Almond	*Prunus dulcis* Mill.	Supernova	Gamma rays (30 Gy)	Italy	1987
Apple	*Malus pumila* Mill.	Mori-hou-fu 3A	Gamma rays (30 Gy)	Japan	1963
		Senbatsu-Fuji-2-Kei	Gamma rays (60 Gy)		1985

Common Name	Botanical Name	Variety	Mutagen Source	Country	Year of Registration
		Belrene	EMS		1970
		Blackjoin BA 2 520	Gamma rays (50 Gy)	France	1970
		Courtagold			1972
		Courtavel			1972
		Lysgolden			1972
		Donghenghongpingguo	Gamma rays (250 Gy)	China	1987
		Dovar	X-rays (30–35 Gy)	Netherlands	1978
		Golden Haidegg	Gamma rays (50 Gy)	Austria	1986
		James Grieve Double Red	Gamma rays (62 Gy)	Czech Republic	1995
		McIntosh 8F-2-32	Gamma rays	Canada	1970
		Shamrock	Gamma rays	Canada	1986
Apricot	*Prunus armeniaca* L.	Early Blenheim	thermal neutrons (thN)	Canada	1970
Banana	*Musa paradisiaca* L.	Klue Hom Thong KU1	Gamma rays (25 Gy)	Thailand	1985
		Novaria	Gamma rays (60 Gy)	Malaysia	1995
		AL-BEELY	Gamma rays	Sudan	2007
		Pirama 1	Gamma rays (30 Gy)	Indonesia	2019
		Fuxuan 01	Gamma rays	China	2005
Clementina	*Citrus celementina* L.	Nero	Fast neutron (6 Gy)	Spain	2006
		Neufina			2010
		CLEMENVERD	Fast neutron (5 Gy)	Spain	

Common Name	Botanical Name	Variety	Mutagen Source	Country	Year of Registration
Ficus	*Ficus benjamina* L.	Golden King	X-rays (25 Gy) AND Gamma rays (20 Gy)	Belgium	1980
		Golden Princess			
Fig	*Ficus carica* L.	Bol (Abundant)	Gamma rays (50–70 Gy)	Russian Federation	1979
Grapefruit	*Citrus paradisi* Macf.	Rio Red	Thermal neutrons (thN)	United States	1970
		Star Ruby			1984
Indian Jujube	*Ziziphus mauritiana* Lamk.	Dao tien	MNH (0.02–0.04%)	Viet Nam	1986
		Ma hong			
Japanese pear	*Pyrus pyriforia* Nak.	Gold Nijisseiki	Gamma rays (0.12–0.15 Gy)	Japan	1991
		Kotobuki Shinsui	Gamma rays (80 Gy)		1997
		Osa Gold			1997
Lemon	*Citrus limon* L.	Eureka 22 INTA	X-rays (10 Gy)	Argentina	1987
Loquat	*Eriobotrya japonica* L.	Shiro-mogi	Gamma rays (200 Gy)	Japan	1982
Mandarin	*Citrus reticulata* L.	Zhongyu 7	Gamma rays (100 Gy)	China	1985
		Zhongyu 8			1986
		Hongju 420			
		NIAB Kinnow	Gamma rays (20 Gy)	Pakistan	2017
		PAU Kinnow-1	Gamma rays (30 Gy)	India	2017
Mulberry	*Morus alba* L.	Sangfu 1	Gamma rays (75 Gy)	China	1974
		Fuzaofeng	Gamma rays (5 Gy)		1992
		Ji 7681	N2 laser		1988
		Fusang 10	Gamma rays		1980

Common Name	Botanical Name	Variety	Mutagen Source	Country	Year of Registration
		Shansang 871	Gamma rays (60 Gy)		1994
		Shigu 11-6	Gamma rays (100 Gy)		1995
		Lala Berry	Colchicine	Japan	2003
		Pop Berry	Colchicine		2004
		S54	EMS	India	1974
Orange	*Citrus sinensis* L.	Hongju 418	Gamma rays (100 Gy)	China	1983
		Xuegan 9-12-1			
		Valencia 2 INTA	X-rays (20 Gy)	Argentina	1987
		IAC 2014	Gamma rays (40 Gy)	Brazil	2016
Papaya	*Carica papaya* L.	Pusa nanha	Gamma rays (150 Gy)	India	1987
Peach	*Prunus persica* L.	Magnif 135	Gamma rays	Argentina	1968
		Shaji 1	CO_2 laser	China	1985
		Shaji 2			
		Fuku-ekubo	Gamma rays (30 Gy)	Japan	1996
		Shimizu Hakutou RS			2004
		Plovdiv 6	Gamma rays (10 Gy)	Bulgaria	1981
Pear	*Pyrus communis* L.	Fuxiangyanghongdli	Gamma rays (2.5 Gy)	China	1983
Plum	*Prunus*	Chaofu 1	Gamma rays	France	1989
		Chaofu 10			
		Chaofu 10			
		Chaofu 2			
		Spurdente-Ferco			1988

Common Name	Botanical Name	Variety	Mutagen Source	Country	Year of Registration
	domestica L.				
Pomegranate	*Punica granatum* L.	Karabakh	Gamma rays (50–70 Gy)	Russian Federation	1979
		Khyrda			
		Plodorodnaya Michurina	X-rays		1977
Sour cherry	*Prunus cerasus* L.	Karlik Samorodka	Gamma rays	Russian Federation	1979
		Polukarlik Orlovskoi Rannei			
		Polukarlik Turgenevki			
		Nishina Zao (DT2008)	Ion beams	Japan	2009
Sweet cherry	*Prunus avium* L. [15]	Compact Lambert	X-rays (40 Gy)	Canada	1964
		Compact Stella 35B-11			1974
		Van 2D-14-11			1972
		Lapins	X-rays		1983
		Lambert 2B-17-18-EC	X-rays (50 Gy)		1972
		Stella			1968
		Stella 16A-7			1972
		Sunburst			1983
		Sumste Samba	Gamma rays[16]		2000
		ALDAMLA [17]	Gamma rays (25 Gy)	Turkey [18]	2014
		BURAK	Gamma rays (50 Gy)		
		Burlat C1	Gamma rays	Italy	1983
		Nero II C1			
		Ferrovia spur	X-rays (4 Gy)		1992
		Super 6	Colchicine	Japan	1997

dment or ransgenic e in vitro uit crops recurrent rise to a practical it still left id itself or types of d breaks e and the ese DSBs ation over e process

equence level, the indel d from natural variations and/or irradiated or chemical mutants [19]. Four SDN-based GE techniques include meganucleases, zing-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the most recent clustered regularly interspaced short palindromic repeat/CRISPR-associated proteins (CRISPR/Cas), respectively. These GE

Common Name	Botanical Name	Variety	Mutagen Source	Country	Year of Registration
		Roman Nishiki			2002

(through and their homozygosity is achieved thro ations or recurrent backcrosse ntroduced mutations. Nevertheless, this is not a case with site-directed or targeted mutagenesis using GE, and GE shared another edge that is its multiplexing capabilities—i.e., simultaneous targeting of multiple genes or copies of a gene. This characteristic can be extremely useful to target homologous genes in polyploid fruit trees. The recent advancements in GE to substitute a single nucleotide allows individual base swapping in a DNA strand [20]. These developments in GE can help to overcome the GMO regulatory frameworks because it leaves no trace of a transgene or exogenous source in the targeted genome. Moreover, there is evidence that the remnants of *Agrobacterium* T-DNA have a role in the evolution of some plant families [21]. Thus, the boundary between natural and engineered crop species may become more blurred after such evidence and ultimately gain the attention of the scientific community to revise the regulatory framework, at least for GE crops.

2. Tree Breeding under Climate Change

The response of trees to any global climate change scenario is a pressing question for natural vegetation and man-made plantations [22]. Climate change is a major threat to tree plantations due to fluctuations in annual precipitation, drought, heat, salinity, and enhanced insect infestations [23]. Investigations to explore the ability and genetic basis of adaptation to global climate change in ecologically and industrially distinct tree species to cope with abiotic and biotic stresses are key research lines in plant science [24]. However, this knowledge has rarely been translated into conserving the genetic resources or bringing the genetic improvements to woody perennials.

The objective of most tree breeding programs is to gradually improve tree populations through recurrent selection cycles and verifications [25]. Traditionally, tree breeding mostly relies on phenotypically selecting superior candidates from the natural or planted stands. It constitutes the base population and further selection builds a pool of selected population with elite donors. Furthermore, these selected populations are then tested for progeny trials and the reselection of parents [26]. However, such selections may cause genetic erosions in the overall populations due to inbreeding depression. The production of hybrids and subsequent backcrossing may accelerate classical breeding with the aim of harnessing heterotic effects by virtue of dominance or over-dominance, tree adaptability and increased yield [27]. Among other potential applications, hybrid breeding has been widely applied to maximize the tree crown perimeter, tree height, conferring resistance to *Fusarium* spp. [28], and to chestnut blight from wild donor tree plants into American chestnut populations [29].

The most promising alternative approach towards tree breeding is molecular- marker-assisted selection (MAS) and molecular-assisted breeding (MAB) [30]. MAS and MAB tools can be effective in pyramiding simple Mendelian traits regulated by a few genes but have limited utility for selection against complex genetic traits in trees [31]. Moreover, MAS and MAB cannot be effective due to fluctuations in allelic frequencies over generations and therefore cannot explain genetic variations for complex traits [1]. To circumvent these limitations, the use of the genomic selection (GD) approach is suitable rather than phenotypic selection-based traditional breeding using MAS and MAB. Despite having a relatively short history, the technique has been successfully implemented in plant breeding. It can

substantially reduce the long breeding cycles for tree breeding and positively enhance the genetic gain over time [31]. In the GS approach, a large number of molecular markers are used to analyze the cumulative effects of QTLs evenly distributed over the genome. Therefore, it makes GS much more efficient for tree breeding due to the possibility to assess the individual genomic estimated breeding value (GEBV) of a single plant. It involves four basic steps: (a) phenotyping and genotyping of the selected individuals from a breeding population, (b) generation of genomic prediction models, (c) model validation on the test population, and d) prediction of GEBV for non-phenotyped individuals and further selection [32]. Unlike MAS, there are no pre-requisites in GS for a prior information about marker linkage, or QTL localizations in the genome and their relative phenotypic effects [33].

3. Mutagenesis as a Source of Genetic Variability in Tree Plants

Genetic improvement through conventional breeding necessarily requires recurrent selection cycles in fruit trees [6] (**Figure 3**). However, a major limitation is the large number of crosses and the development of subsequent filial generations. This is even more challenging in fruit trees such that recurrent selection may take decades of continuous breeding efforts [34]. The lengthy breeding process can be accelerated in fruit frees with more advanced techniques such as MAS and GWAS for QTLs [35]. For example, many quality- and yield-related traits have been improved in apple, banana, mandarin, peach, and strawberry through conventional breeding coupled with mutagenesis, MAS, genetic engineering, MAB and others [6][7][9]. The genetic improvements in fruit trees are, however, progressing at a slower pace, but the availability of pangenomes, broader understanding of genotypic and phenotypic interactions and fast-track breeding may hasten the development of fruit tree cultivars with better genetic makeup.

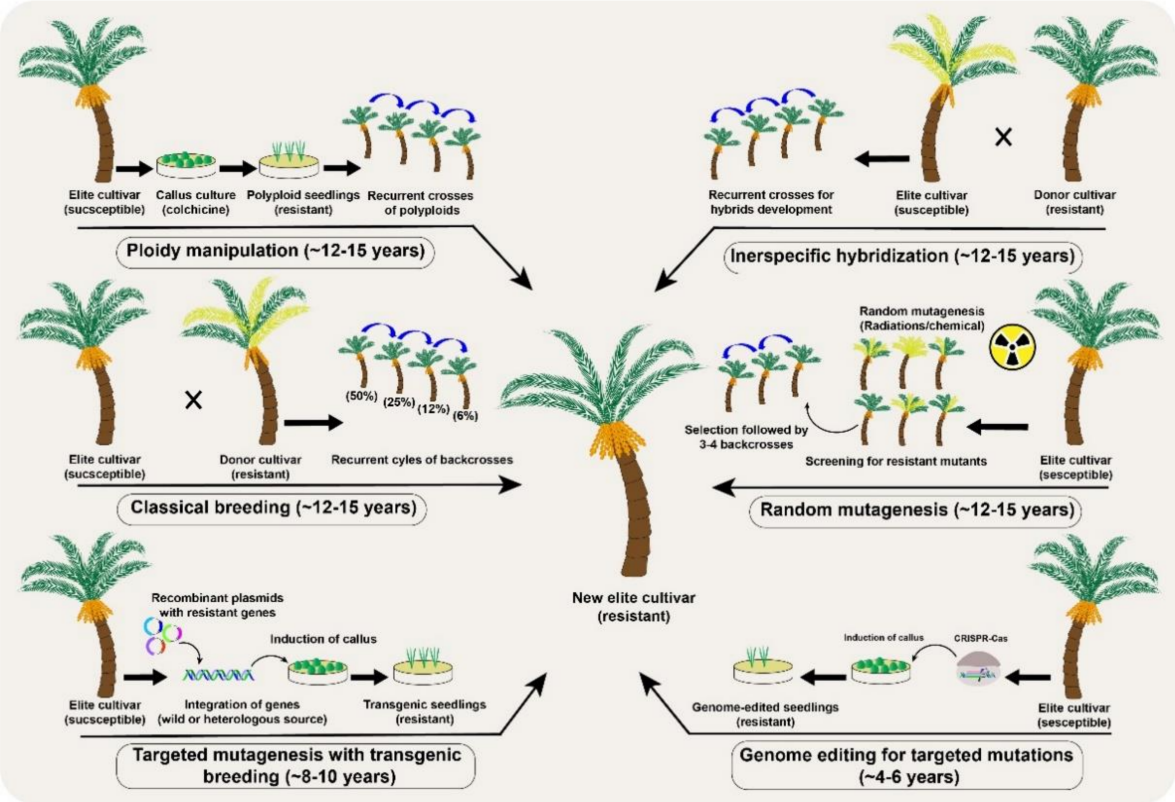

Figure 3. A comparative analysis of different conventional and the new breeding tools (NBTs) to modify desirable genetic modifications in a date palm (*Phoenix dactylifera* L.) fruit crop.

Genetic improvement through conventional breeding is limited to sexually compatible crop plants [36]. Nevertheless, the genetic diversity of self-incompatible plants can be increased by mutagen treatment (physical or chemical) to induce new mutations in cultured cells, seeds, seedlings, or sometimes whole plants. Random mutations are preferred in seeds because the small number of cells in the developing embryo makes mutagenesis much easier and produces fewer chimeric plants [37]. During somatic mutations, a fewer number of cells or limited sectors in the apical meristem are affected, creating chimeric mutated plants. Such sectorial mutations involve genetic differences in either one or two layers of cells and is more frequent in vegetatively propagated fruit trees [38]. An effective way to dissociate chimerism in vegetatively propagated plant is through organogenesis or embryogenesis [39][40]. The mutation frequency and population structure of the mutants directly depend upon the type of mutagen and the time of exposure. Irrespective of the used mutagens, the ultimate induced mutations are random and therefore require a large screening population to identify the desired mutants [41]. Mutation breeding is advantageous over conventional breeding because it precludes segregation progenies while improving the genetic make-up during selection cycles.

High energy X-ray radiations were the earliest mutagens used to induce desired traits in fruit trees [37]. Currently, X-ray mutagenesis has been either replaced or supplemented with other more advanced physical mutagens such as fast neutrons, ionizing radiations and gamma rays. Besides bringing about beneficial mutations from single-nucleotide replacements to chromosomal aberrations, these mutagens may trigger DNA damage directly or indirectly in the form of oxygen radicals [42]. Physical mutagenesis has been successfully used to improve a

number of traits in fruit trees—for example, improved heat tolerance in pineapple [43], self-fertile in cherry fruits, fruit color in apple, bunch size in banana, short-statured papaya plants, disease resistant pear and growth earliness in grapevine [44].

Among the chemical mutagens, ethylmethanesulfonate (EMS) is the most widely employed alkylating agent in fruit crops [45], including banana and peach [46]. However, it is not suitable for vegetatively propagated fruit trees and perennial allogamous fruit trees because of their heterozygous genomes and prolonged life cycle. Although, chemical mutagens are extremely efficient in inducing desirable mutagenesis in whole plants or seeds, it is not recommended for tissue-cultured plants due to their extreme toxicity [37]. Chemical mutagens predominantly cause hemizygous point mutations and successive backcrosses are necessary to obtain a homozygous line and to stabilize the mutated gene of interest [47]. On the other hand, physical mutagenesis has a high risk of a collateral effect on non-targeted genes because the impact of physical mutagens produces multisite mutations of various sizes. For example, the use of fast neutron mutagenesis causes large deletions in the genome besides translocations and chromosomal loss [48][49]. Chemical mutagens are more affordable; however, these carry serious health and environmental risks. Moreover, chemically induced mutations are genetically less stable than physical mutations.

Polyploidy is another interesting natural phenomenon in plant evolution, adaptation, and speciation, which can also be induced using colchicine, for genetic improvements. Colchicine application induces autopolyploidy by blocking mitosis without interfering with DNA replication and ultimately doubles the chromosome numbers (**Figure 3**). The generation of triploid dessert apple and tetraploid grapevine cultivars are successful examples of autopolyploids in fruit crops [50]. Interspecific hybridizations have also been tested in some citrus cultivars, including the formation of natural hybrids [51]. However, as in conventional breeding, if the hybrids are fertile in perennial fruit trees, multiple backcrosses are still needed to remove the undesirable genetic background of the recessive parent. For example, scab resistance in apple took more than 40 years [52], and the enhancement of sugar and antioxidants levels in elderberries took at least 10 years through the interspecific hybridization approach [53].

Somaclonal variation is a natural phenomenon occurring during in vitro tissue culturing and can produce useful genetic variations in plants [54]. It includes DNA-related genetic or epigenetic variations, which induce phenotypic changes distinguishable from the original parent. Major causes include but are not limited to prolonged in vitro culturing, tissue culturing media composition, the presence of phytohormones and certain other mechanical factors during culturing [55]. Somaclones can be detected through morphological assessments of the off-type regenerants, biochemical response of explants, fingerprinting with protein or isozymes-based markers, and cytogenetic assessment [56][57]. In addition, more advanced DNA- or transposon-based molecular markers [58] and the use of next-generation sequencing (NGS) screening have also been successfully applied to detect somaclonal variations in fruit tree breeding.

TILLING as a Powerful Tool in Mutation Breeding

Numerous significant genes from older mutant cultivars continue to serve as a foundation for modern gene pools in commercial cultivars. Nonetheless, the burden of unwanted genetic mutations and the development of new breeding tools (NBTs) have had an effect on the use of random mutation techniques [59]. Recent advancements in screening methods enable the detection of SNPs and complex traits at the molecular level, which are otherwise difficult to discern with conventional screening methods. The utilization of mutagenesis underwent a huge change with the development of TILLING (targeting induced local lesions in genomes) as a high-throughput mutant screening technique to identify point mutations at a specific locus in the mutated genome [60]. The TILLING technique redirected mutation breeding away from laborious forward genetics approaches to reverse genetics approaches, allowing plant breeders to detect mutations in known genes. Furthermore, TILLING has been accompanied with the more advanced next-generation sequencing (NGS) techniques to provide more practical solutions to bypass extensive mutant screening for the selected genes [61].

The major mutation screening methods in TILLING include celery nuclease (CEL I) [62], high-resolution melting (HRM) [63] and NGS [64]. The mismatch-specific CEL I method is a popular TILLING technique, which is coupled with the LI-COR gel analyzer system. The HRM incorporates the PCR technique in which the monitoring of dsDNA product is monitored with a dsDNA-specific fluorescent dye followed by the formation of a high-resolution melting curve. The more advanced NGS technique has further facilitated the mutant screening in a TILLING population through whole-genome sequencing, de novo assembly and resequencing tools.

The basic procedure of TILLING includes mutation induction through chemical, physical or biological agents to produce an M1 population. These M1 plants are then allowed to self-pollinate and generate M2 plants. Total genomic DNA is isolated and subjected to eightfold DNA pooling followed by PCR amplification of the gene of interest. The recurrent heating and cooling steps form heteroduplexes, which are then incubated with CEL I endonuclease to cleave mismatches in these heteroduplexes. The cleaved DNA products are separated on a denaturing gel electrophoresis and the fluorescence is detected with a LI-COR DNA analyzer. The induced mutations are then verified by sequencing of the polymorphic individuals, respectively [65]. Although the CEL I -based TILLING platform has been widely used, the critical steps such as enzymatic digestions, cloning and gel electrophoresis make it time consuming. Moreover, insufficient genome sequence information of many plant species affects the efficacy of this TILLING platform [66]. Contrarily, the HRM-based TILLING offers more accurate, sensitive, and cost-effective mutant screening through PCR and analysis of the DNA melting curve. Nevertheless, detection of small insertions and deletions is difficult and limited to amplicons with a size <450 bp with HRM [67]. The NGS-based TILLING platform is comparatively a potential screening method with more accurate mutant screening. However, the high cost, the generation of a large sequencing dataset and the requirement of sophisticated bioinformatics tools still pose major challenges to its adoption in studying the genetics and genomics of mutagenic studies [65].

4. Genomics and Genetic Engineering Perspectives of Trees

The genetic improvement of the tree plant genome can be accelerated through two distinct approaches: MAB through quantitative trait loci (QTL) mapping, and direct gene transfer through genetic engineering. The whole

genomes of many tree plants have been completely sequenced; consequently, comprehensive genetic architecture of useful genetic traits are now available, which can be helpful for marker-assisted breeding, MAB [68][69]. The availability of such datasets can widely assist in genetic expression, and functional and comparative genomics. Moreover, recent developments in –omics and NGS technologies and, in parallel, more advanced bioinformatics tools, can expedite in-depth molecular studies in trees [70][71]. The transcriptomic, proteomic and metabolomics data sets of woody plants are dynamically bridging the gaps between tree genomes and genetic expression studies.

Genetic transformation can be improvised by inserting single or multiple genes directly into the elite background across the species or genus without long cycles of selections and screening [72][73], e.g., herbicide tolerance in populous [74]. The first application of genetic engineering in fruit trees was in papaya when papaya varieties ‘Sunset’ and ‘Kapoho’ were genetically modified by inserting the capsid protein (CP) gene of papaya ringspot virus (PRSV) to confer viral resistance. Consequently, the first transgenic papaya cultivar was developed in 1998 [75]. Recently, the USA approved a non-browning arctic apple cultivar [76][77]. Several other genetic traits for fruit quality, tree physiology and abiotic stress tolerance have been successfully engineered for transgenic apple, banana, papaya, and pineapple [73][75]. Current transgenic fruit trees approved in the USA include papaya against PRSV, plum against plum pox virus (PPV) [78], apple with the non-browning trait [79] and pineapple cultivar ‘Pinkglow’ [80]. Transgenic papaya plants have been successfully engineered to alter elite traits related to tree growth, nitrogen metabolism, lignin contents and abiotic stress tolerance [81][82]. Moreover, resistance in papaya was also conferred against phytophthora blight, papaya dieback disease (PDBD) and papaya ringspot virus (PRSV) in several studies [83]. Among non-transgenic approaches, dsRNA-mediated protection strategies have also been practiced in papaya against PRSV [84]. Similarly, eucalyptus species have also been genetically transformed to introduce genes from endogenous or heterologous sources to modify their salt tolerance status and secondary cell wall constituents [85]. Many pine softwood tree species have also been utilized for transgenic developments for various traits [69].

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